# Purine Analog Inhibitors of Xanthine Oxidase - Structure Activity Relationships and Proposed Binding of the Molybdenum Cofactor

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A number of new hypoxanthine analogs have been prepared as substrate inhibitors of xanthine oxidase. Most noteworthy inhibitory new hypoxanthine analogs are 3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**47**), $ID_{50}$ 0.06 $\mu M$ and 3-phenylpyrazolo[1,5-*a*]pyrimidin-7-one (**46**), $ID_{50}$ 0.40 $\mu M$. 5-(*p*-Chlorophenyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**63**) and the corresponding 5-nitrophenyl derivative **64** exhibited an $ID_{50}$ of 0.21 and 0.23 $\mu M$, respectively. 7-Phenylpyrazolo[1,5-*a*]-*s*-triazin-4-one (**40**) is shown to exhibit an $ID_{50}$ of 0.047 $\mu M$. The structure-activity relationships of these new phenyl substituted hypoxanthine analogs are discussed and compared with the xanthine analogs 3-*m*-tolyl- and 3-phenyl-7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-ones (**90**) and (**91**), previously reported from our laboratory to have $ID_{50}$ of 0.025 and 0.038 $\mu M$, respectively. The presence of the phenyl and substitutedphenyl groups contribute directly to the substrate binding of these potent inhibitors. This work presents an updated study of structure-activity relationships and binding to xanthine oxidase. In view of the recent elucidation of the pterin cofactor and the proposed binding of this factor to the molybdenum ion in xanthine oxidase, a detailed mechanism of xanthine oxidase oxidation of hypoxanthine and xanthine is proposed. Three types of substrate binding are viewed for xanthine oxidase. The binding of xanthine to xanthine oxidase is termed *Type I* binding. The binding of hypoxanthine is termed *Type II* binding and the specific binding of alloxanthine is assigned as *Type III* binding. These three types of substrate binding are analyzed relative to the most potent compounds known to inhibit xanthine oxidase and these inhibitors have been classified as to the type of inhibitor binding most likely to be associated with specific enzyme inhibition. The structural requirements for each type of binding can be clearly seen to correlate with the inhibitory activity observed. The chemical syntheses of the new 3-phenyl- and 3-substituted phenylpyrazolo[1,5-*a*]pyrimidines with various substituents are reported. The syntheses of various 8-phenyl-2-substituted pyrazolo-[1,5-*a*]-*s*-triazines, certain *s*-triazolo[1,5-*a*]-*s*-triazines and *s*-triazolo[1,5-*a*]pyrimidine derivatives prepared in connection with the present study are also described.

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## Introduction.

Our interest in xanthine oxidase inhibitors, extending over a ten-year period, has been outlined in an earlier publication [1]. The synthesis of a series of nitrogen heterocycles resembling in general the natural substrates for xanthine oxidase, *i.e.* hypoxanthine and xanthine has provided a unique opportunity to examine in detail the binding of various heterocyclic moieties to the enzyme, xanthine oxidase. The present structure-activity relationships are presented in the hope that this work may serve as a prototype study of a rather detailed investigation of purine like nitrogen heterocyclic systems as they bind to specific enzymes. Most of the compounds here studied were synthesized as potential analog substrates which would bind to xanthine oxidase but would not be metabolized to a different product such as a nucleoside, nucleotide or oligonucleotide, thereby hopefully avoiding interaction with other enzymes involved in purine metabolism which might result in undue toxicity.

Allopurinol (pyrazolo[3,4-*d*]pyrimidin-4-one, **1**), despite certain disadvantages [2], is currently the drug of choice, world-wide, for the treatment of hyperuracemia and gouty arthritis. Allopurinol was synthesized and first reported by Robins [3] and shortly thereafter by Schmidt and Druey [4]. Feigelson, Davidson and Robins [5] first showed that derivatives of the pyrazolo[3,4-*d*]pyrimidine ring system were potent inhibitors of xanthine oxidase. For all enzymatic studies here described, xanthine oxidase isolated from bovine milk was employed. The details of the assay procedures have been described in our earlier publication

[1]. The $I_{50}$ values (the concentration of compound that produces a 50% inhibition of the uninhibited control reaction) were determined in $\mu M$, using xanthine as a substrate. Under these conditions, the $I_{50}$ of allopurinol **1** was 5.9 $\mu M$ and the $I_{50}$ of pyrazolo[3,4-*d*]pyrimidine-4,6-dione (alloxanthine or oxoallopurinol, **79**) was 2.3 $\mu M$, which corresponds to the value reported by Baker, Wood and Kozma [6]. This is in the same range as for the isoguanine

Table I

Hypoxanthine, Xanthine and Guanine Analogs as Inhibitors of Xanthine Oxidase

| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
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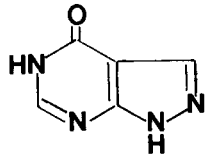
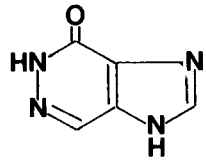
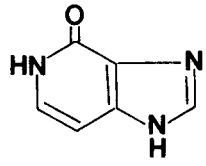
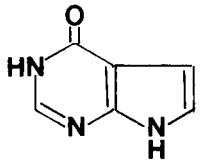
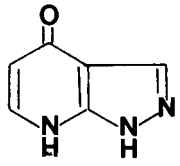
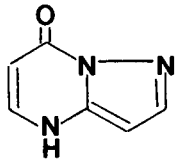
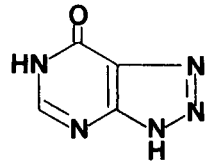
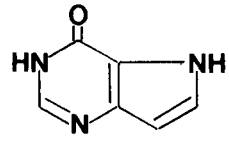
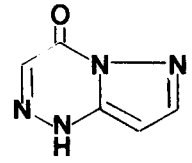
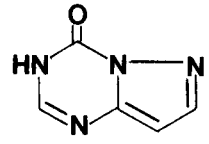
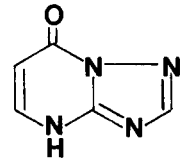
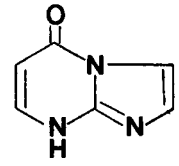
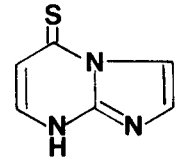
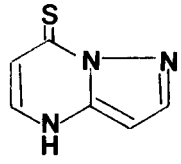
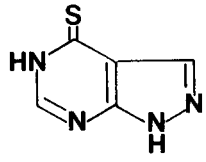
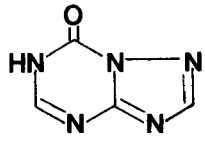
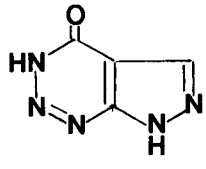
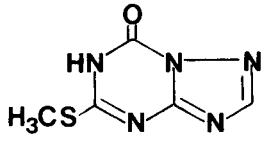
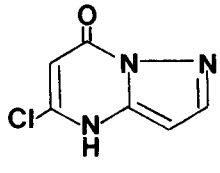
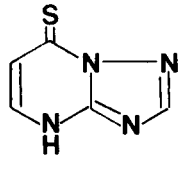
| 1. | II |  | 5.9 | 3 |
| 2. | |  | >150 | 69 |
| 3. | |  | 98 | 70 |
| 4. | |  | 110 | 71 |
| 5. | |  | 130 | 56 |
| 6. | I |  | 11 | 12 |

| No. | Type of Binding | Compound | $I_{50}$ μM | Synthesis and Related Reference |
|---|---|---|---|---|
| 7. | I |  | 8.2 | 72 |
| 8. | |  | 270 | 73 |
| 9. | |  | >140 | 74 |
| 10. | |  | 84 | 13 |
| 11. | |  | 45 | 16 |
| 12. | |  | 120 | 14 |
| 13. | II |  | 5.4 | 15 |

| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 14. | I |  | 70 | 57 |
| 15. | II |  | 8.4 | 3,6 |
| 16. | I |  | 80 | 63 |
| 17. | III |  | 7.4 | 10 |
| 18. | I |  | 1.4 | a |
| 19. | I |  | 24 | a |
| 20. | I |  | 54 | 16 |

| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
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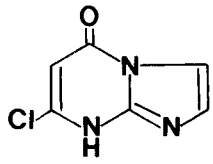
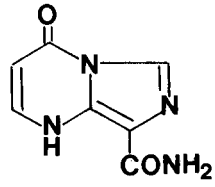
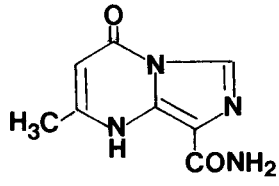
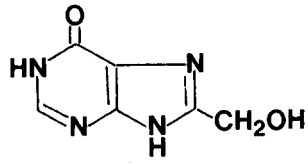
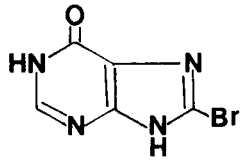
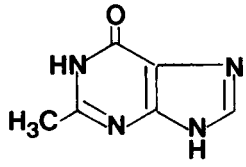
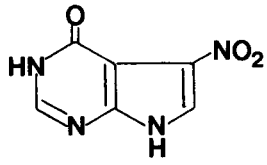
| 21. | |  | >150 | 14 |
| 22. | |  | 0.9 | a |
| 23. | |  | >140 | 75 |
| 24. | I |  | 6.6 | 76 |
| 25. | |  | 120 | a |
| 26. | |  | >150 | 77 |
| 27. | II |  | 0.40 | 71 |

| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
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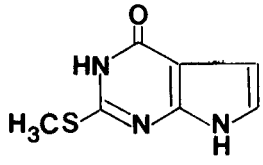
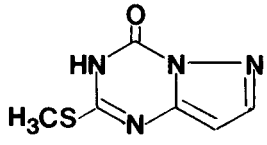
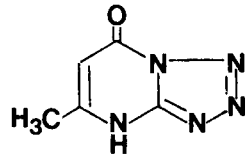
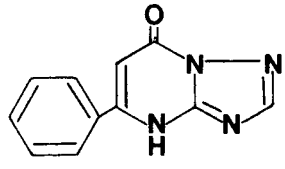
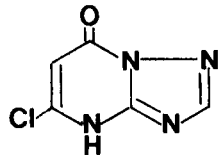
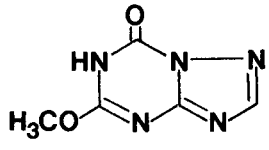
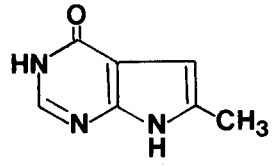
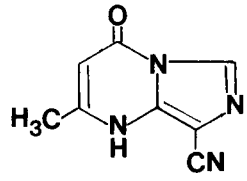
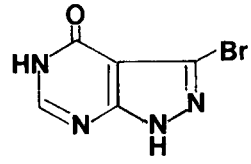
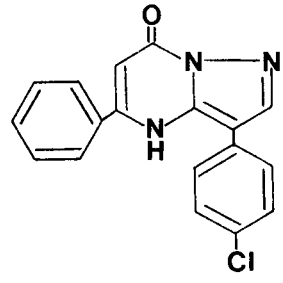
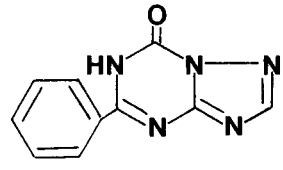
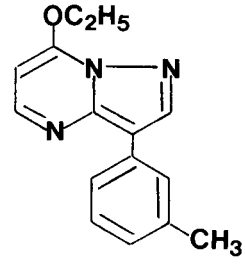
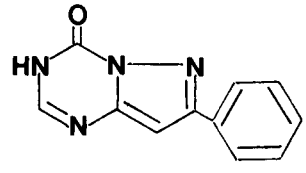
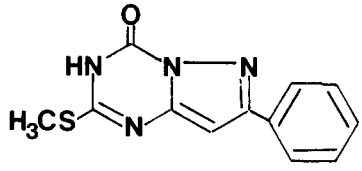
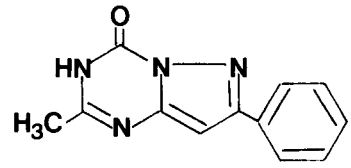
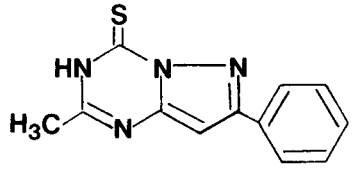
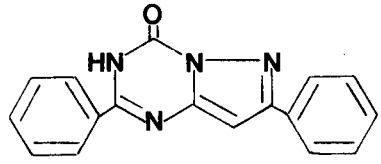
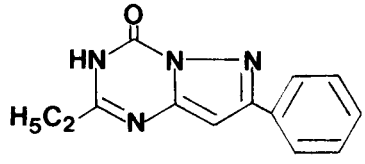
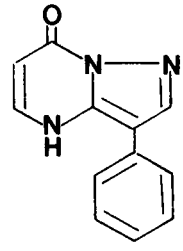
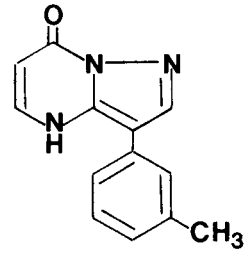
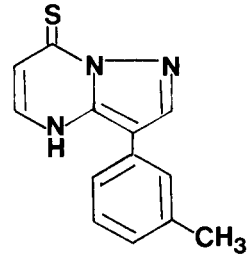
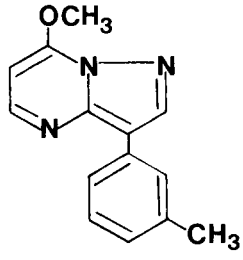
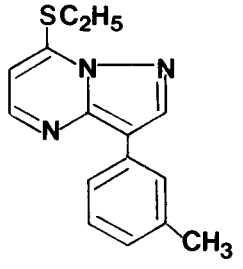
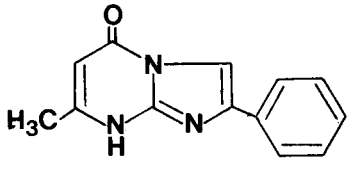
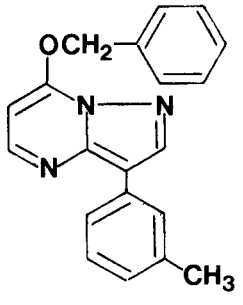
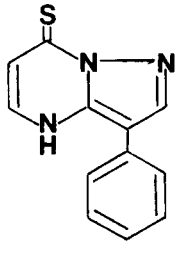
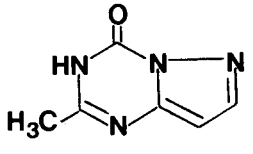
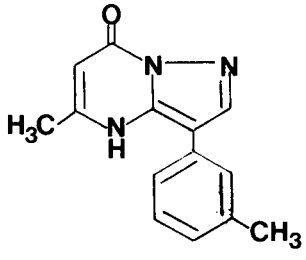
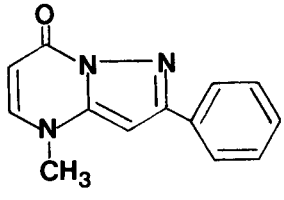
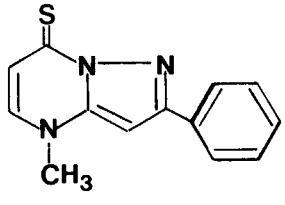
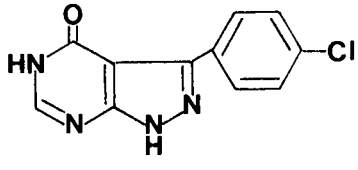
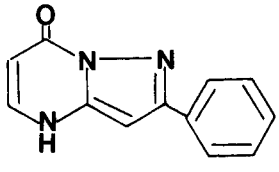
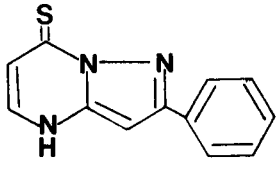
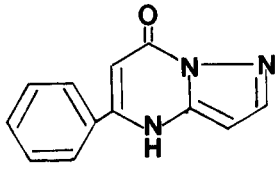
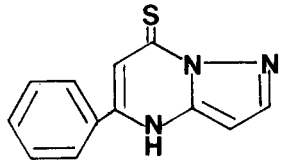
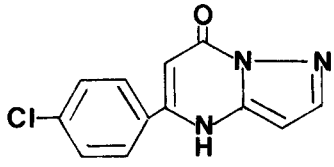
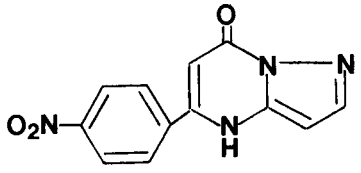
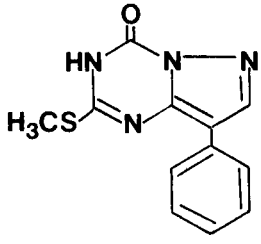
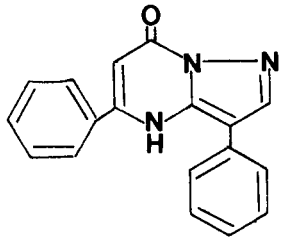
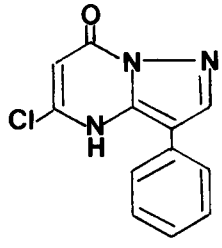
| 28. | |  | >120 | 78 |
| 29. | |  | 130 | 13 |
| 30. | |  | >150 | 79 |
| 31. | I |  | 21 | a |
| 32. | |  | 78 | 65 |
| 33. | |  | 80 | a |
| 34. | |  | 45 | a |

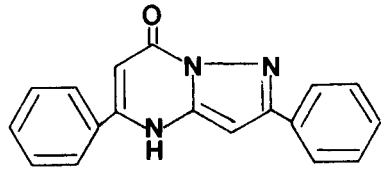
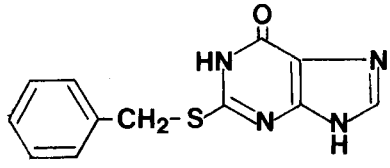
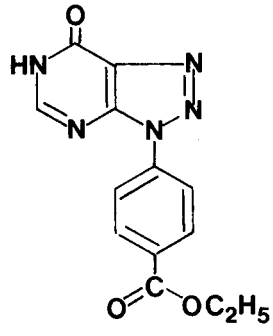
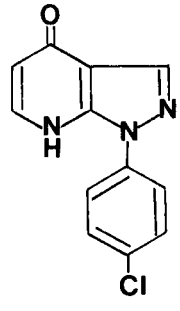
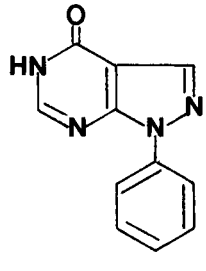
| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 35. | |  | 110 | 75 |
| 36. | II |  | 23 | 11 |
| 37. | |  | 40 | 57 |
| 38. | |  | 64 | a |
| 39. | |  | >140 | a |
| 40. | I |  | 0.047 | 19 |
| 41. | |  | 160 | 80 |

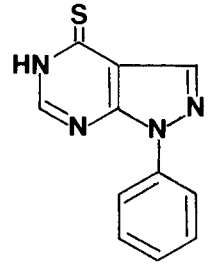
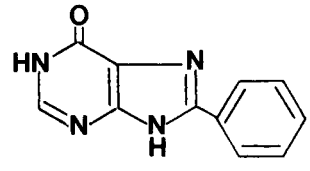
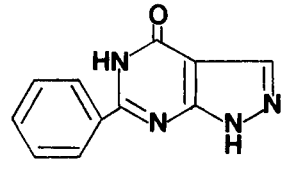
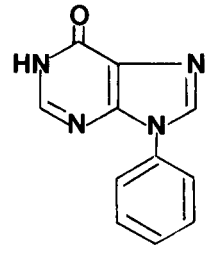
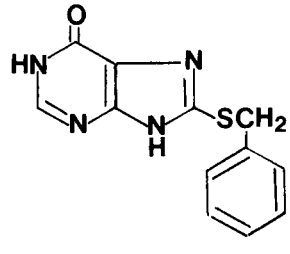
| No. | Type of Binding | Compound | $I_{50}$ μM | Synthesis and Related Reference |
|---|---|---|---|---|
| 42. | |  | 80 | 81 |
| 43. | |  | >150 | 58 |
| 44. | |  | 110 | a |
| 45. | |  | 110 | a |
| 46. | I |  | 0.40 | 56 |
| 47. | I |  | 0.06 | a |
| 48. | I |  | 0.40 | 57 |

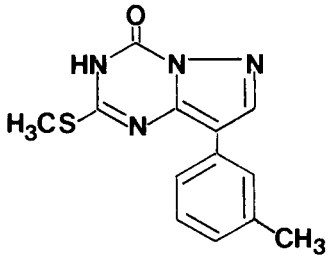
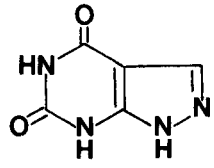
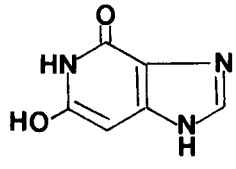
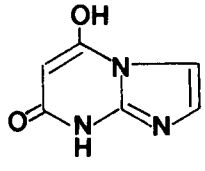
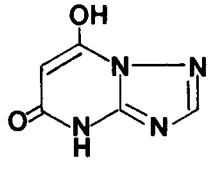
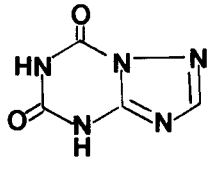
| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 49. | |  | 150 | a |
| 50. | |  | 110 | a |
| 51. | |  | >120 | 82 |
| 52. | |  | 17 | a |
| 53. | I |  | 0.58 | 57 |
| 54. | |  | 110 | a |

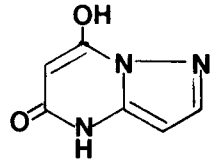
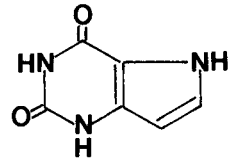
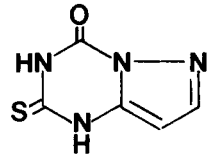
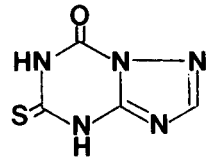
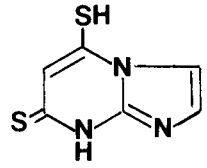
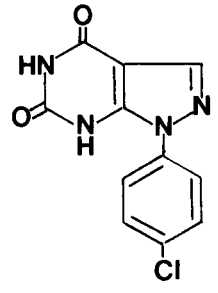
| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 55. | |  | >140 | 57 |
| 56. | |  | >150 | 57 |
| 57. | |  | >150 | 57,83 |
| 58. | III |  | ~ 70 | 20 |
| 59. | I |  | 50 | 56,84 |
| 60. | I |  | 5.8 | 57 |
| 61. | I |  | 0.40 | 57 |

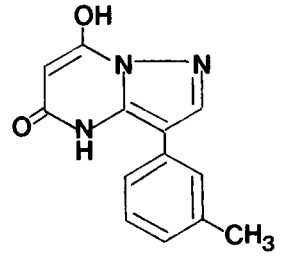
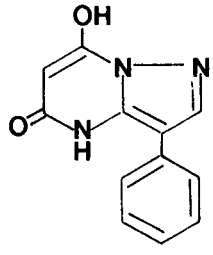
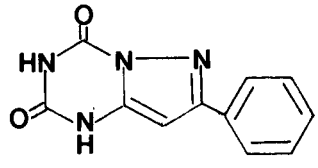
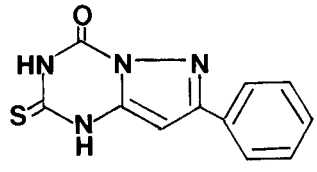
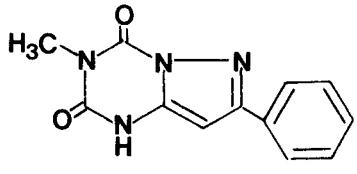
| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 62. | I |  | 5.1 | 57 |
| 63. | I |  | 0.21 | a |
| 64. | I |  | 0.23 | a |
| 65. | |  | >140 | a |
| 66. | |  | 91 | 57 |
| 67. | |  | 93 | a |

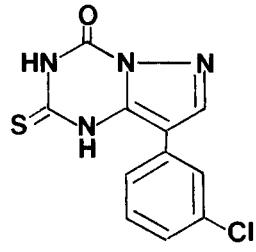
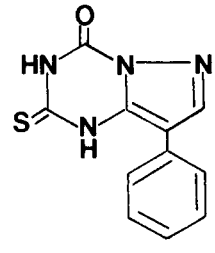
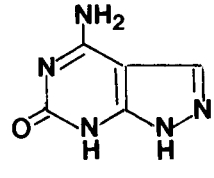
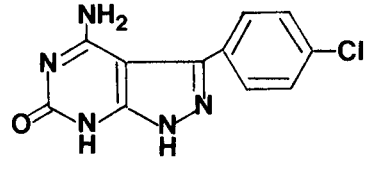
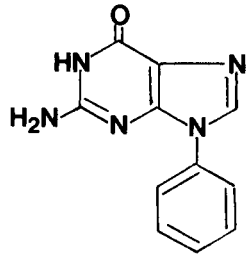
| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 68. | |  | 160 | 57,85 |
| 69. | II |  | 0.75 | 17 |
| 70. | I |  | 3 | 86 |
| 71. | |  | 108 | 11 |
| 72. | |  | 260 | 6,87 |

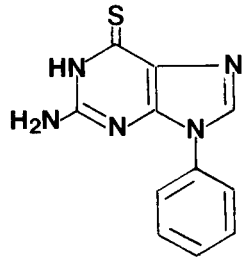
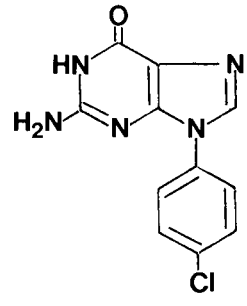
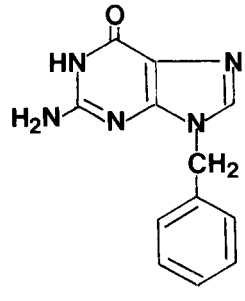
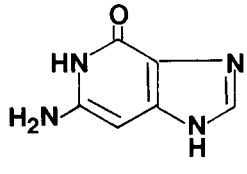
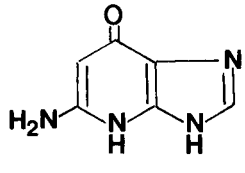
| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 73. | |  | 88 | 6,87 |
| 74. | I |  | 0.062 | 6 |
| 75. | II or III |  | 6.5 | 6,88 |
| 76. | I |  | 13 | 89 |
| 77. | I |  | 2.8 | 6,17 |

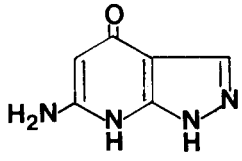
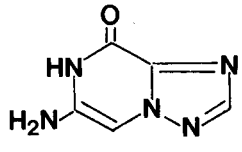
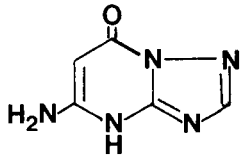
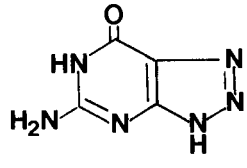
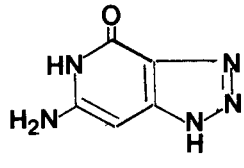
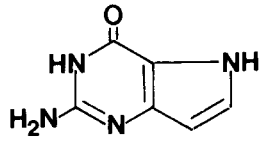
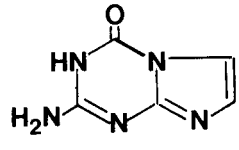
| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 78. | |  | >140 | a |
| 79. | III |  | 2.3 | 3,6 |
| 80. | |  | >150 | 90 |
| 81. | II |  | 36 | 24 |
| 82. | |  | >150 | 91 |
| 83. | I |  | 45 | a |

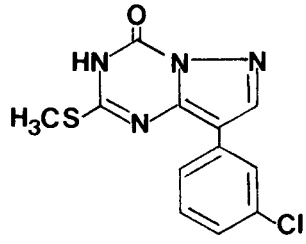
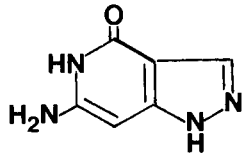
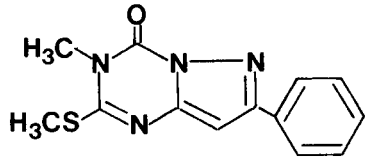
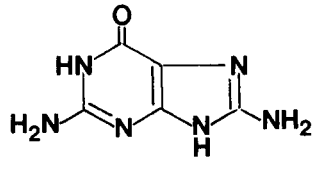
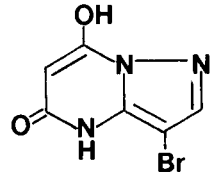
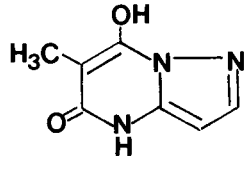
| No. | Type of Binding | Compound | $I_{50}$ μM | Synthesis and Related Reference |
|---|---|---|---|---|
| 84. | I |  | 4.5 | 23 |
| 85. | |  | >300 | 92 |
| 86. | |  | 160 | 13 |
| 87. | |  | 100 | 62 |
| 88. | |  | 54 | 15 |
| 89. | |  | 0.57 | 6,93 |

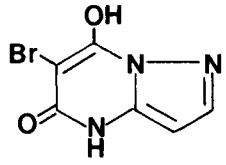
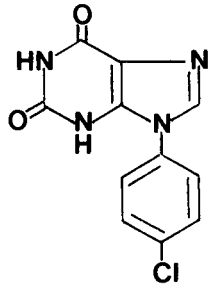
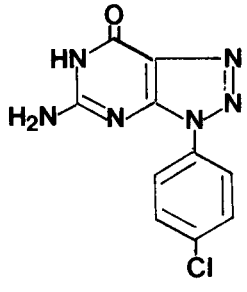
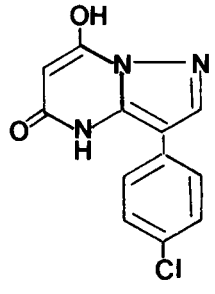
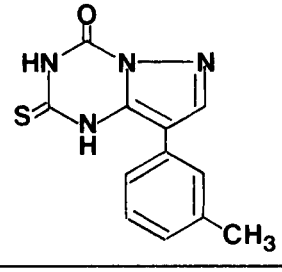
| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 90. | I |  | 0.025 | 1 |
| 91. | I |  | 0.038 | 1 |
| 92. | I |  | 37 | 80 |
| 93. | |  | 160 | 80 |
| 94. | |  | 43 | 80 |

| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 95. | I |  | 11 | a |
| 96. | |  | 140 | a |
| 97. | III |  | 2.0 | 3,5 |
| 98. | III |  | ~ 5 | 20 |
| 99. | I |  | 0.41 | 29,94 |

| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 100. | I |  | 1.1 | 29,94 |
| 101. | I |  | 1.8 | 29,94 |
| 102. | I |  | 23 | 29,94 |
| 103. | I |  | 93 | 27 |
| 104. | I |  | 23 | 95 |

| No. | Type of Binding | Compound | $I_{50}$ μM | Synthesis and Related Reference |
|---|---|---|---|---|
| 105. | |  | >120 | 96 |
| 106. | |  | 56 | 97 |
| 107. | |  | 73 | a |
| 108. | I |  | 14<br>7.5 | 17,72 |
| 109. | I |  | 38 | 28 |
| 110. | |  | 7.3 | 25 |
| 111. | |  | 23 | 26 |

| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 112. | |  | 110 | a |
| 113. | |  | 84 | 98 |
| 114. | |  | >150 | 80 |
| 115. | I |  | 16 | 99 |
| 116. | I |  | 1.2 | 1 |
| 117. | |  | 54 | 1 |

| No. | Type of Binding | Compound | $I_{50}\mu M$ | Synthesis and Related Reference |
|---|---|---|---|---|
| 118. | I |  | 9.2 | 1 |
| 119. | |  | 240 | 6,94 |
| 120. | I |  | 0.25 | 29,94 |
| 121. | I |  | 0.043 | 1 |
| 122. | |  | 110 | a |

[a] Synthesis described in the present paper.

analog 4-aminopyrazolo[3,4-*d*]pyrimidin-6-one (**97**) which was reported [5] to have an $ID_{50}$ of 1-2 $\mu M$ in the first published account of the pyrazolo[3,4-*d*]pyrimidines tested as inhibitors of xanthine oxidase. In the present assay the value determined for **97** was 2 $\mu M$.

Baker and co-workers [6], using 1-(*p*-chlorophenyl)pyrazolo[3,4-*d*]pyrimidine-4,6-dione (**89**), prepared in our laboratory [7], showed that the presence of the *p*-chlorophenyl group at position 1 increased the binding efficiency of alloxanthine five-fold to give an $ID_{50}$ of 0.57 $\mu M$. Similarly, Baker and co-workers [6] showed that 9-(*p*-chlorophenyl)-guanine (**101**) [7] exhibited an $ID_{50}$ of 1.8 $\mu M$ against xanthine oxidase. These and related studies by Baker and Wood [8] suggested that hydrogen at position 9 of guanine was unnecessary in the complexing to xanthine oxidase and that there is a considerable increase in binding due to a hydrophobic phenyl group at $N^9$. Baker and Wood [8] attempted to map this hydrophobic binding area. We have similarly shown that the presence of a phenyl group at the 3-position of 7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-one (**91**), increases the $ID_{50}$ of the parent xanthine analog over 130 times to give an $ID_{50}$ for **91** of 0.038 $\mu M$, which is 155 times more potent as an inhibitor than allopurinol [1]. In the present study we also report that 3-phenylpyrazolo-[1,5-*a*]pyrimidin-7-one (**46**) exhibits an $ID_{50}$ of 0.4 $\mu M$. The present work covers a rather extensive study of the structure-activity relationships of additional analogs of hypoxanthine, xanthine and guanine including appropriate phenyl and substituted phenyl substituents on various heterocyclic ring systems involving 5-membered rings fused to six-membered azole systems *via* a bridgehead nitrogen atom. A number of new and very potent inhibitors of xanthine oxidase are here reported for the first time. Several of these inhibitors are excellent candidates for further studies with a view toward clinical trial. These inhibitors of xanthine oxidase are of special interest in view of the recent findings that xanthine oxidase inhibitors such as allopurinol may indeed prove useful in inhibiting intestinal lesions following hemorrhagic shock and intestinal ischemia [9]. Such lesions are believed to be due to superoxide generated by the excess presence of xanthine oxidase [9] in the intestines during shock.

Structure-Activity Study of Hypoxanthine Analogs as Inhibitors of Xanthine Oxidase.

Table I lists 78 hypoxanthine analogs prepared and studied as xanthine oxidase inhibitors. The inhibition is expressed as $ID_{50}$ in $\mu M$. As previously noted [6], pyrazolo-[3,4-*d*]pyrimidine-4-thione (**15**) [2] is nearly as potent an inhibitor as allopurinol. Loss of the pyrimidine nitrogen at $N^5$ of allopurinol as in **5** results in considerable loss of potency. Similarly, loss of $N^3$ of hypoxanthine results in loss of binding as in the hypoxanthine analog **3**. Loss of $N^9$ of hypoxanthine appears to be considerably more serious than loss of $N^7$ (compare **8** *vs* **4**). The insertion of a nitrogen at position 6 in allopurinol gives pyrazolo[3,4-*d*]-*v*-triazin-4-one (**17**) [10], which retains most of the potency of allopurinol (Table I). 8-Bromohypoxanthine (**25**) is considerably less potent than 8-azahypoxanthine (**7**). The introduction of a methyl group at position 2 of hypoxanthine gives 2-methylhypoxanthine (**26**), which is essentially an inactive compound. It is of interest, however, that introduction of the polar hydroxymethyl group into position 8 of hypoxanthine gives a compound **24** virtually as active as allopurinol. Replacement of $N^7$ in hypoxanthine by a $C\text{-}NO_2$ group, 5-nitropyrrolo[2,3-*d*]pyrimidin-4-one (**27**) gives a hypoxanthine analog with $ID_{50}$ of 0.40 $\mu M$, while 6-methylpyrrolo[2,3-*d*]pyrimidin-4-one (**34**) is considerably less active. The introduction of a bromo group at the 3 position of allopurinol (**36**), decreases the binding affinity [11]. Rearrangement of the nitrogens in the pyrimidine ring of hypoxanthine would appear to be generally detrimental; for example, compounds **2**, **9** and **21**. Of considerable interest is the fact that pyrazolo[1,5-*a*]pyrimidin-7-one (**6**) [12], a hypoxanthine analog which contains a bridgehead nitrogen, retains most of the inhibitory effect of allopurinol ($ID_{50}$ of 11.0 $\mu M$). The presence of an adjacent nitrogen atom in the six-membered ring, such as in **9** entirely eliminates inhibitor binding. The presence of an additional nitrogen in the six-membered ring adjacent to the keto function as in pyrazolo[1,5-*a*]-1,3,5-triazin-4-one (**10**) [13], also reduces the binding efficiency considerably. It would appear that the presence of fewer nitrogens as in pyrazolo[1,5-*a*]pyrimidin-7-one (**6**), provide more basic nitrogen atoms which probably serve as a better binding site for the enzyme xanthine oxidase. For example, the addition of another nitrogen into the five-membered ring of **6** to give **11** also results in significant reduction of potency. It is noteworthy that imidazo[1,2-*a*]pyrimidin-5-one (**12**) [14], in contrast to **6**, is considerably less potent as an inhibitor, suggesting the $N^7$ of hypoxanthine may serve as a natural substrate binding site. Of interest is the fact that the corresponding thione analog of **6**, pyrazolo[1,5-*a*]pyrimidine-7-thione (**14**) [15] is less active than the corresponding oxo analog **6**, whereas the corresponding imidazo-[1,2-*a*]pyrimidine-5-thione (**13**) is more potent than the similar oxo analog **12** and is essentially as active as allopurinol. The presence of both nitrogens in the five-membered ring as for *s*-triazolo[2,3-*a*]pyrimidine-4-thione (**20**) [16], reduced the inhibitory activity of **13** ten times (Table I). Of interest is the fact that a proper susbstituent in the six-membered ring can be beneficial. For example, 2-benzylthiohypoxanthine (**69**) (Table I) exhibits an $ID_{50}$ of 0.75 $\mu M$ [17]. The similar introduction of a methylthio substituent into the corresponding position of 5-azahypoxanthine (**16**) also increases the inhibitory potency over 50 times as

in the case of **18**. Similarly, an electronegative substituent such as chloro in 5-chloropyrazolo[1,5-*a*]pyrimidin-7-one (**19**) and 7-chloroimidazo[1,2-*a*]pyrimidin-5-one (**21**) results in compounds which are significantly less potent inhibitors. This is viewed as the halogen reducing the basicity of the requisite binding sites on the nitrogens. For example, comparison of the corresponding hypoxanthine analogs **6** *vs* **19**, **32** *vs* **11** and **12** *vs* **21**. Of considerable interest is the fact that the presence of the phenyl group in 8-phenylhypoxanthine (**74**) gives a hypoxanthine derivative with the amazing $ID_{50}$ of 0.062 $\mu M$ [6]. Similarly, 9-phenylhypoxanthine (**76**) [18] is a significant inhibitor of xanthine oxidase [6] with an $ID_{50}$ of 13 $\mu M$. In this regard, in the present study it was visualized that an introduction of a phenyl substituent into the 2 or 3 position (equivalent to the 8 or 9 position of hypoxanthine) could considerably increase the inhibitory potency of pyrazolo[1,5-*a*]pyrimidin-7-one (**6**). This indeed proved to be the case since 3-phenylpyrazolo[1,5-*a*]pyrimidin-7-one (**46**) showed an $ID_{50}$ of 0.40 $\mu M$ and the corresponding *m*-toluyl derivative **47** exhibited an $ID_{50}$ of 0.06 $\mu M$, which proved to be one of the most potent hypoxanthine analogs studied. The 2-phenylpyrazolo-[1,5-*a*]pyrimidin-7-one (**59**) was less potent ($ID_{50}$ of 50 $\mu M$), however, the corresponding 2-phenylpyrazolo[1,5-*a*]pyrimidine-7-thione (**60**) was approximately as active as allopurinol ($ID_{50}$ of 5.8 $\mu M$). Transfer of the phenyl ring to position 5 as in the case of **61** provided a compound with $ID_{50}$ of 0.40 $\mu M$, again considerable binding due to the hydrophobic phenyl group. The corresponding 5-(p-chlorophenyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**63**) and the 5-*p*-nitrophenyl derivative (**64**) are even more potent with $ID_{50}$ of 0.21 and 0.23 $\mu M$, respectively. An attempt to combine the enhancement of the potency of the phenyl group at positions 3 and 5 was made in the synthesis of 3,5-diphenylpyrazolo[1,5-*a*]pyrimidin-7-one (**66**) and 3-(*p*-chlorophenyl)-5-phenylpyrazolo[1,5-*a*]pyrimidin-7-one (**37**). These heterocycles, however, proved to be less active than the parent hypoxanthine analog **6** or its 3-phenyl derivative **46**. It would thus appear that the monophenyl derivatives **46** and **61** may be oriented to give binding to xanthine oxidase so as to get maximum hydrophobic interaction of the monophenyl substituent. However, 2,5-diphenylpyrazolo[1,5-*a*]-pyrimidin-7-one (**68**) is considerably less potent than 2-phenylpyrazolo[1,5-*a*]pyrimidin-7-one (**59**). It is most interesting to note that 2-phenylpyrazolo[1,5-*a*]pyrimidine-7-thione (**60**) and 5-phenylpyrazolo[1,5-*a*]pyrimidine-7-thione (**62**) are approximately equal in inhibitory potency to allopurinol, whereas 3-phenylpyrazolo[1,5-*a*]pyrimidine-7-thione (**53**) is considerably more potent with $ID_{50}$ of 0.58 $\mu M$. Thus the phenyl group at position 3 appears to provide the maximum binding enhancement of this ring system. It is noteworthy that the presence of a methyl group at position 5 as in the case of **55** greatly reduces the inhibitory activity. Similar observation was made with compounds **55** and **47**. Loss of the oxo or thio function at position 7 by alkylation on the oxygen or sulfur greatly reduced the inhibitory potency (*e.g.* **39** and **49** *vs* **47** and **50** *vs* **48**). It is of interest, however, that a bulky substituent on the 7-oxygen, such as benzyl (compound **52**) has a tendency to restore activity. 5-Chloro-3-phenylpyrazolo[1,5-*a*]pyrimidin-7-one (**67**) is considerably less active than **46** which suggests that the presence of the chlorine reduces the basicity of the adjacent $N^4$ nitrogen which may serve as a binding site. Also alkylation at $N^4$ as in the case of **56** and **57** essentially eliminated the activity of **59** and **60**, respectively, suggesting that this nitrogen may be a binding site for xanthine oxidase in the pyrazolo[1,5-*a*]pyrimidin-7-one ring system. Success achieved with the pyrazolo[1,5-*a*]pyrimidine ring suggested a similar study of the pyrazolo-[1,5-*a*]-1,3,5-triazine ring system, which involves the addition of another nitrogen to the six-membered ring. The synthesis of 7-phenylpyrazolo[1,5-*a*]-*s*-triazin-4-one (**40**) [19], resulted in a *very* potent hypoxanthine analog with an $ID_{50}$ of 0.047 $\mu M$. Thus, **40** is 1,780 times more potent than the parent pyrazolo[1,5-*a*]-*s*-triazin-4-one (**10**). Thus **40** is the most potent hypoxanthine analog inhibitor studied in our laboratory so far. Introduction of a methyl, ethyl or methylthio group at position 2 greatly reduced the binding (*e.g.* **42**, **45** and **41**). Introduction of an additional phenyl group at position 2 resulted in considerable loss of activity (compound **44**), similar to the results found with the pyrazolo[1,5-*a*]pyrimidine series. A methylthiosubstituent at position 2 plus a methyl group at $N^3$ (compound **114**) completely eliminates the activity of **40**. 2-Methylthio-7-arylpyrazolo[1,5-*a*]-*s*-triazin-4-ones (**65** and **78**) are only weakly active. 2-Methylthio-5-azahypoxanthine (**18**) has an $ID_{50}$ of 1.4 $\mu M$, which is superior to the activity of 5-azahypoxanthine (**16**) ($ID_{50}$ of 80 $\mu M$). 2-Methoxy-5-azahypoxanthine (**33**) is less active than **18** and is approximately equal in potency to 5-azahypoxanthine (**16**) itself. The introduction of a phenyl group at position 2 to give **38** increased the activity only slightly over **18**. Comparison of the activity of **38** with that of **31** and **61** shows that the introduction of additional nitrogen atoms into the 5-membered ring (**31**, $ID_{50}$ of 21 $\mu M$) or the six-membered ring (**38**, $ID_{50}$ of 64 $\mu M$), greatly reduces the binding affinity (**61**, $ID_{50}$ of 0.40 $\mu M$), presumably by reducing the basicity of nitrogens $N^1$ and $N^4$ in **61**.

Unique is the potent inhibitory activity of imidazo[1,5-*a*]-pyrimidin-4-one-8-carboxamide (**22**) which exhibited $ID_{50}$ of 0.9 $\mu M$. In this instance, it would appear that the carboxamide group may participate directly in some manner in binding to xanthine oxidase. The introduction of a methyl group at position 2 to give 2-methylimidazo[1,5-*a*]pyrimidin-4-one-8-carboxamide (**23**) virtually abolished the activity. The corresponding 2-methyl-8-cyanoimidazo[1,5-*a*]pyrimidin-4-one (**35**) was also inactive.

The presence of a phenyl group in the 3-position of allo-

purinol, 3-(*p*-chlorophenyl)allopurinol (**58**) [20] reduces the potency about ten-fold and gives a compound about as active as 1-phenylpyrazolo[3,4-*d*]pyrimidine-4-thione (**73**). The presence of a phenyl group at the 8-position of hypoxanthine gives a compound with an $ID_{50}$ of 0.062 $\mu M$ and corresponds to the highly active isomeric 7-phenylpyrazolo[1,5-*a*]-1,3,5-triazin-4-one (**40**) [19]. Thus the position 8 of hypoxanthine would appear to be the best position for phenyl group binding. The other alkylamino substituted compounds (**125-131, 137-140**) synthesized during this study were devoid of any xanthine oxidase inhibitory activity.

Structure-Activity Study of Xanthine and Guanine Substrate Analogs as Inhibitors of Xanthine Oxidase.

Table I also lists 43 xanthine and guanine analogs (**79** to **122**) which have been studied as inhibitors of xanthine oxidase. The standard for these studies is pyrazolo[3,4-*d*]pyrimidine-4,6-dione (**79**), first synthesized by Robins [3] and reported in 1956. Allopurinol is slowly oxidized to oxipurinol (**79**) by xanthine oxidase, which then binds **very** tightly to the enzyme [21]. The $Mo^{IV}$ apparently complexes to the two pyrazole nitrogens in an enzyme complex which dissociates only very slowly [21,22]. Of a number of simple xanthine analogs studied, 7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-one (**84**) [23] was found in our laboratory [1] to be of the same order of inhibition as allopurinol. Insertion of an additional nitrogen into the 5-membered ring of **84** at position-3 to give **82** resulted in a loss of inhibitory activity. 5-Azaxanthine (**83**) was found to be ten times less active than **84**. The xanthine analog 5-hydroxyimidazo[1,2-*a*]pyrimidin-7-one (**81**) and 3-deazaxanthine (**80**) were much less active than **84**. 9-Deazaxanthine (**85**) is essentially without inhibitory effect. In most instances, introduction of a thio group for one or both of the oxo functions resulted in loss of activity of the xanthine analogs. For example, compare **83** *vs* **87**; **81** *vs* **88**. 3-Bromo-7-hydroxypyrazolo[1,5-*a*]pyrimidine-5-one (**116**) [1] was found to be more potent as an inhibitor than **84**. 6-Bromo-7-hydroxypyrazolo[1,5-*a*]pyrimidine-5-one (**118**) [24] exhibited an $ID_{50}$ of 9.2 $\mu M$ compared to 4.5 $\mu M$ for the parent xanthine analog **84**. 6-Methyl-7-hydroxypyrazolo[1,5-*a*]pyrimidine-5-one (**117**) [24] was considerably less active than **84**.

Of the simple guanine analogs, 8-azaguanine (**108**) and 9-deazaguanine (**110**) [25] are most inhibitory with an $ID_{50}$ of 14 and 7.3 $\mu M$, respectively. 1-Deazaguanine (**104**) and 2-aminoimidazo[1,2-*a*]-*s*-triazin-4-one (**111**) [26], both exhibit an $ID_{50}$ of 23 $\mu M$. 3-Deazaguanine (**103**) [27] and 3-deaza-8-azaguanine (**109**) [28], show an $ID_{50}$ of 93 and 38 $\mu M$, respectively. 9-(*p*-Chlorophenyl)guanine (**101**) reported from our laboratory [7] shows an $ID_{50}$ of 1.8 $\mu M$ [29]. 6-Aminopyrazolo[4,3-*c*]pyridin-4-one (**113**) and 5-amino-*s*-triazolo[1,5-*a*]pyrimidin-7-one (**107**) were much less active with an $ID_{50}$ of 84 and 73 $\mu M$, respectively. 8-Aminoguanine (**115**) is nearly as active as 8-azaguanine (**108**) ( $ID_{50}$ of 14 $\mu M$ as compared to 16 $\mu M$). Other guanine analogs, **105** and **106** were not particularly potent inhibitors.

9-Phenylguanine (**99**) and 9-phenyl-6-thioguanine (**100**), prepared in our laboratory [7], were first shown by Baker [29] to be excellent inhibitors of xanthine oxidase with an $ID_{50}$ of 0.41 and 1.1 $\mu M$ [6], respectively. 9-Benzylguanine (**102**) [7] proved to be less active [6] ($ID_{50}$ of 23 $\mu M$). The most active guanine analog presently reported is 9-(*p*-chlorophenyl)-8-azaguanine (**120**) [7] with an $ID_{50}$ of 0.25 $\mu M$ [29].

Since the *p*-chlorophenyl group at position 1 greatly increased the potency of pyrazolo[3,4-*d*]pyrimidine-4,6-dione (**79** *vs* **90**), it was decided to introduce the *p*-chlorophenyl group at the 3-position of 7-hydroxypyrazolo[1,5-*a*]-pyrimidin-5-one (**84**) to give compound **121**, which was found [1] to exhibit an $ID_{50}$ of 0.043 $\mu M$. A series of 3-substitutedphenyl-7-hydroxypyrazolo[1,5-*a*]pyrimidin-5-ones with comparable potency have been prepared and reported [1] (*e.g.* see compounds **90, 91** and **121**). These xanthine analogs are more potent than the corresponding 9-phenylxanthine derivative (compare **121** *vs* **119**) or the corresponding 1-(*p*-chlorophenyl)pyrazolo[3,4-*d*]pyrimidine-4,6-dione (compare **121** *vs* **89**). 2-(*m*-Chlorophenyl)-5-thiopyrazolo[1,5-*a*]-*s*-triazin-7-one (**95**) exhibits an $ID_{50}$ of 11 $\mu M$, whereas the corresponding 2-phenyl- (**93**) and 3-phenyl-(**96**) derivatives gave an $ID_{50}$ of 160 and 140 $\mu M$, respectively. It is interesting that 9-(*p*-chlorophenyl)xanthine (**119**), in contrast to 9-(*p*-chlorophenyl)guanine (**101**), is relatively inactive. The introduction of a *p*-chlorophenyl group into the 3-position of 4-aminopyrazolo[3,4-*d*]pyrimidin-6-one, the isoguanine analog (**97**), gives **98** with about half of the inhibitory activity of **97** (Table I). It is interesting that often the activity of the hypoxanthine analogs carry over to the corresponding xanthine analogs in the same ring system. For example, the activity of **1** and **79**; **46** and **91**; **47** and **90**; **6** and **84**.

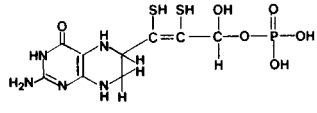
Substrate and Inhibitor Binding to the Molybdenum Cofactor of Xanthine Oxidase.

Allopurinol (**1**) is a clinically effective, well tolerated drug in the treatment of hyperuricemia. Side effects are few, which include leukopenia, thrombocytopenia, renal impairment, dermatitis, rashes and mild gastrointestinal distress [30,31]. Allopurinol and oxoallopurinol (alloxanthine, **79**) are converted by purine nucleoside phosphorylase to the 1-ribosyl derivatives [32]. Oxoallopurinol is also converted by uridine phosphorylase to the 7-ribosyl derivative [32] which has been isolated in the urine of patients treated with allopurinol. Oxoallopurinol nucleotides have been found to be potent inhibitors of orotidine 5'-phosphate decarboxylase with 1-$\beta$-D-ribofuranosyloxoallopurinol being

the most inhibitory [33]. These data strongly suggest that such inhibition is responsible for the large amounts of orotate and orotidine excreted in the urine of patients treated with allopurinol [33,34]. Allopurinol has been demonstrated to have an immunosuppressive effect when mice with skin grafts have been treated daily with 200 $\mu$g [35]. Because of the possible effects of these nucleoside and nucleotide derivatives which could be deleterious after long periods of usage of allopurinol, efforts to find more potent inhibitors of xanthine oxidase but lacking potential for nucleoside and nucleotide formation could provide a product without the observed undesirable side effects.

Massey and co-workers [21,36,37] reported that allopurinol is first oxidized to oxoallopurinol which then binds very tightly to the reduced molybdenum xanthine oxidase cofactor in a complex which is believed to be largely responsible for the *in vivo* inhibitory action of allopurinol. These studies have recently been reviewed [36]. The binding has been proposed to occur with the molybdenum$^{IV}$ cofactor and the pyrazole nitrogens of oxoallopurinol with both pyrazole nitrogens interacting directly with Mo$^{IV}$ [22]. In 1974, Olson and co-workers [38] proposed a model for the oxidation of xanthine to uric acid based on Mo$^{IV}$ cofactor binding on N$^7$ of xanthine followed by a disulfide anion attacking at C$^8$, which is displaced by a water molecule to give uric acid and Mo$^{IV}$. Bray and co-workers [39] later suggested molybdenum binding to N$^9$ of xanthine and proton abstraction from C$^8$ *via* a thiol group directly attached to molybdenum. Spector [40] has recently proposed a modification of the model where a nucleophile X$^-$ attached to the molybdenum cofactor **first** attacks C$^8$, followed by molybdenum $^{VI}$ attachment to N$^9$, followed by hydrogen ion transfer to the sulfur attached directly to molybdenum which picks up the electron pair from N$^9$.

The molybdenum cofactor of xanthine oxidase has recently been isolated in purified form and has been shown to consist of molybdenum and a reduced form of pterin [41]. The molybdenum cofactor complex is noncovalently attached to protein [42]. The isolation of pterin-6-carboxylic acid was achieved after oxidation of the cofactor with alkaline permanganate [41-44]. More recently, a complete structure of the molybdenum cofactor has been proposed as I, based on a series of extensive studies [45]. The American [43,44] and Russian authors [45] propose that

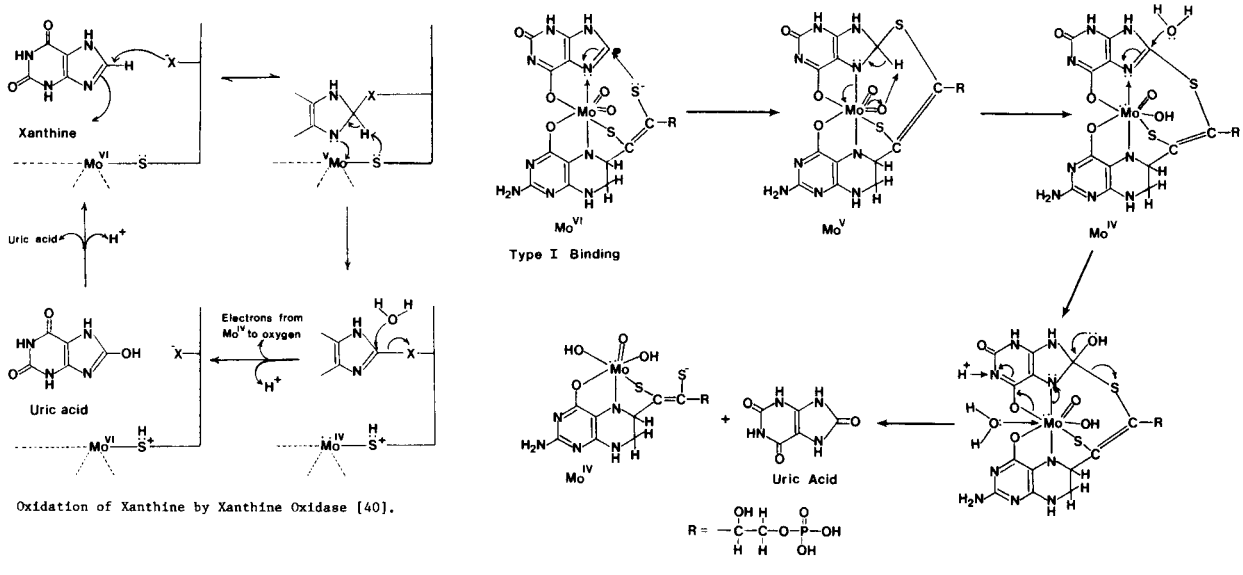


I

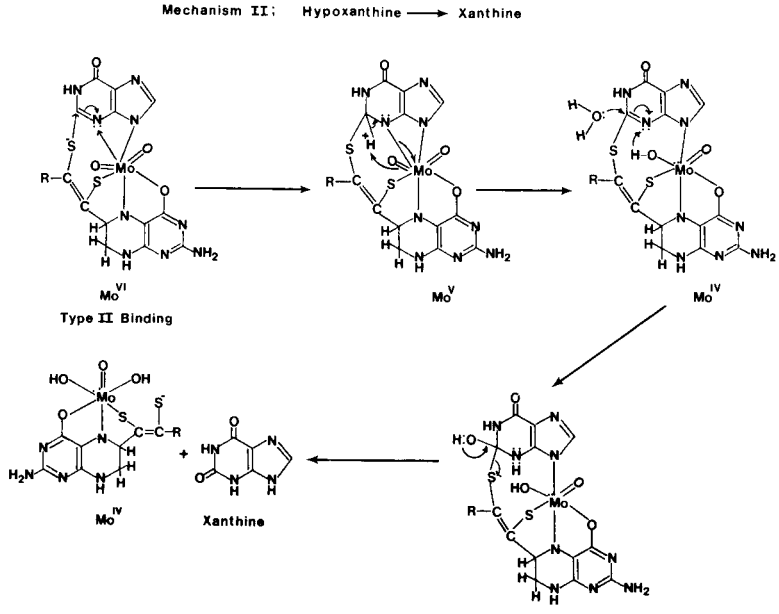
the molybdenum is attached to the oxygen of the pyrimidine ring, the nitrogen at N$^5$ and the sulfur on the carbon attached to C$^6$ of the pterin moiety. Using these concepts the oxidation of xanthine by xanthine oxidase may now be visualized as follows:

It is proposed that the xanthine oxidase Mo$^{VI}$ cofactor binds xanthine at O$^6$ and N$^7$ to give a binding which we shall call *Type I Binding*. The positive charge at N$^7$ now makes the 8 position vulnerable to nucleophilic attack by the neighboring group sulfide ion. Abstraction of the C$^8$ proton, presumably by a molybdenum oxygen, releases the electron pair on N$^7$ to the molybdenum to give Mo$^{IV}$, which may bind again (or stay bonded to N$^7$ by simply transferring the electrons to Mo) in the Mo$^{IV}$ state. Uric acid is now formed by attack of water at C$^8$ followed by elimination of the sulfide group and regeneration of the keto function at C$^6$ and the cofactor which rapidly transfers the electron pair and returns to the Mo$^{VI}$ cofactor to repeat the process (see *Mechanism I*). The process of generation of xanthine from hypoxanthine is visualized to occur *via*

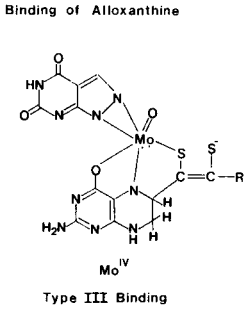
Mechanism I; Xanthine ⟶ Uric Acid




Oxidation of Xanthine by Xanthine Oxidase [40].




$Mo^{VI}$ cofactor binding at $N^9$ and $N^3$ in a binding pattern which we shall call *Type II Binding*. A third type of binding of the Mo cofactor to purinone type analogs can be illustrated by the binding of $Mo^{IV}$ cofactor to alloxanthine which involves very tight binding as noted by Massey [21, 36,37], and Walsh [22]. This we refer to as a *Type III Binding*.




Hawkes and co-workers [46] have recently used EPR to establish binding of the molybdenum to the N-2 nitrogen of alloxanthine. This latter type binding has been noted for the adjacent five membered ring nitrogens for binding and is probably responsible for the extraordinary tight binding of certain pyridyl-1,2,4-triazoles [47,48] which are known to be good inhibitors of xanthine oxidase. Most of the potent inhibitors of xanthine oxidase described in the present work bind by a *Type I* binding to the substrate analog. For example, 3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**47**), should bind as shown in Figure 1. Even though this type of binding is similar to that of xanthine, the greater electron density of the five-membered ring is such

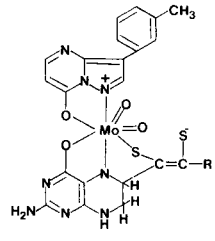


Fig. 1

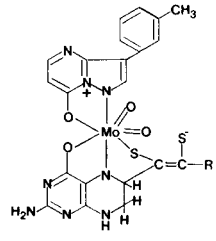


Fig. 1a

as to prevent attack by sulfide ion at the adjacent carbon and the positive charge may be shared by the bridgehead nitrogen (Figure 1a). Thus *no* oxidized product is obtained. The presence of the *m*-tolyl group at position 3 although removed from the binding site, is particularly helpful in increasing lipophylic binding to the enzyme. Other potent inhibitors which appear to bind by *Type I* binding are compounds **6, 14, 31, 40, 46, 48, 53, 61, 62, 63, 64, 70, 74, 77, 84, 90, 91, 92, 95, 108, 116, 118, 120** and **121** (Table I). Substrate analog inhibitors which appear to bind by a *Type II* binding mechanism are **13, 15, 17, 27, 69, 75** and **81**. Substrate analog inhibitors which probably bind by a *Type III* binding mechanism are **17, 58, 75, 79, 97, 98, 108** and **109**. Some substrate analogs such as **17** and **75** could bind as *Type II* or *Type III* and **1, 58, 108** and **115** could bind *via Type I* or *Type III* binding. It is interesting that compounds such as **24, 74** and **77** may bind as per *Type I*, but the presence of the 8-substituent prevents the entrance of the sulfide ion or the lack of a hydrogen for abstraction prevents formation of an oxidized substrate. These 8-substituted purines are excellent inhibitors of xanthine oxidase. Similarly, the potency of **70, 108, 38** and **120** may be viewed as *Type I* binders in which nucleo-

philic substitution at $C^8$ is prevented by the presence of a ring nitrogen at that position.

Inspection of Table I and a study of possible binding types reveals why many of the purine analogs which might otherwise have been expected to be active are inactive because of the lack of ability to bind by one of the three proposed binding types. The loss of inhibitory activity of **39** or **49** over that of **47** is readily explained by the presence of the oxygen alkyl group which prevents *Type I* binding. Similarly, the potency of **48** is lost in **50** due to the ethyl group on sulfur. The relative inactivity of **4, 9, 12, 28, 34, 35, 51, 56, 57, 73** and **94** may be due to a loss of ability for *Type I* binding. The presently proposed *mechanisms I* and *II* are consistent with single crystal X-ray and EPR studies on model systems [49-51]. Most evidence is consistent with Mo cycling through the VI, V and IV oxidation states [51]. The model proposed here is also consistent with protonation of one of the two terminal oxygens upon reduction of the molybdenum [52,53].

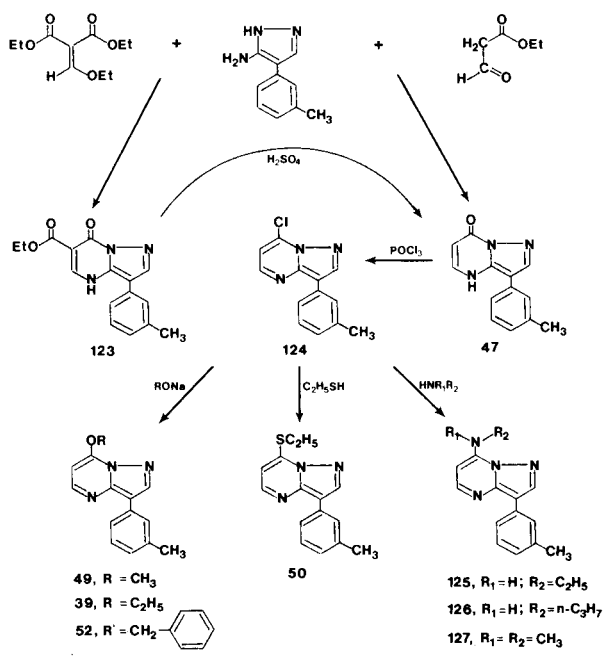
As an example of an analog substrate which appears to have clinical utility, 3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidin-6-one (**47**) was studied further. The acute oral median lethal dose ($LD_{50}$) was determined in albino rats (Charles River strain) to be 15,380 mg/kg. The acute intraperitoneal toxicity in the same strain of rats was determined to be 2,700 mg/kg. The compound **47** was found to have no effect on IMP synthesis. Using the method of Johns *et al.* [54], compound **47** was found not to be a substrate for xanthine oxidase as compared to allopurinol which is an excellent substrate. Compound **47** was also found not to be a substrate for PRPP or HxGPRPP transferase. The half-life of **47** in the plasma of humans after a single oral dose of 200 mg or 400 mg was 12 hours.

Studies of **47** and several of the other potent inhibitors of xanthine oxidase reported here for the first time are continuing with a view to selection of possible candidates for further clinical evaluation.

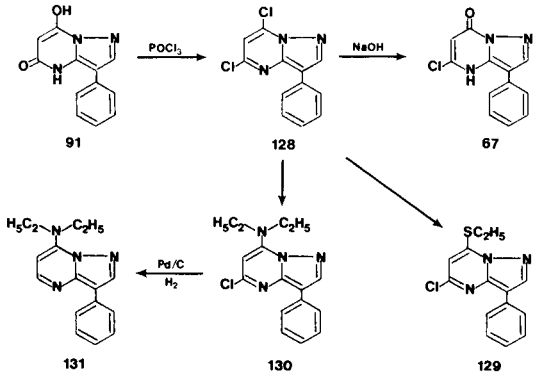
Chemical Syntheses.

A. Pyrazolo[1,5-*a*]pyrimidines.

The synthesis of 3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**47**) was accomplished by treatment of the sodium salt of $\alpha$-formylacetate and 3-amino-4-(*m*-tolyl)pyrazole [55] in refluxing ethanol to give 59% yield of **47**. This procedure is similar to that of Reimlinger *et al.* [56] who have reported the synthesis of 3-phenylpyrazolo[1,5-*a*]pyrimidin-7-one (**46**). 6-Carbethoxy-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**123**) was prepared by the treatment of 3-amino-4-(*m*-tolyl)pyrazole with ethoxymethylenemalonate in refluxing acetic acid according to the general procedure of Makisumi [23]. Treatment of **123** with refluxing 40% sulfuric acid provided another synthetic route to **47** *via* hydrolysis and decarboxylation of the 6-carbethoxy group. Treatment of **47** with refluxing phosphoryl chloride in the presence of *N,N*-dimethylaniline gave an 82% yield of 7-chloro-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**124**). The 7-alkoxy-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidines, the 7-methoxy (**49**), 7-benzyloxy (**52**) and the 7-ethoxy (**39**) derivatives were each prepared by heating **124** with the corresponding sodium alkoxide. Treatment of **124** with ethanethiol in the presence of potassium hydroxide at room temperature gave a 93% yield of 7-ethylthio-3-(*m*-tolyl)pyrazolo-[1,5-*a*]pyrimidine (**50**). Treatment of **124** with various amines in refluxing ethanol gave the corresponding 7-ethylamino (**125**), 7-*N,N*-dimethylamino (**127**) and 7-*n*-propylamino-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**127**), respectively.

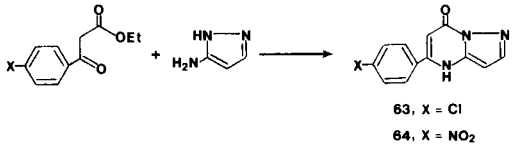


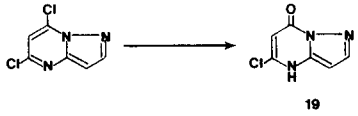

When 3-phenyl-7-hydroxypyrazolo[1,5-*a*]pyrimidine-5-one (**91**) [1] was treated with refluxing phosphoryl chloride in the presence of *N,N*-dimethylaniline, an 80% yield of 5,7-dichloro-3-phenylpyrazolo[1,5-*a*]pyrimidine (**128**) was obtained. When **128** was heated with 5% aqueous sodium hydroxide on a steam bath 5-chloro-3-phenylpyrazolo-[1,5-*a*]pyrimidin-7-one (**67**), was obtained. Treatment of **128** with ethanethiol in the presence of potassium hydroxide at room temperature gave 5-chloro-7-ethylthio-3-phenylpyrazolo[1,5-*a*]pyrimidine (**129**), in 66% yield. Reaction of diethylamine and **128** at room temperature in ethanol gave 5-chloro-7-*N,N*-diethylamino-3-phenylpyrazolo[1,5-*a*]-pyrimidine (**130**). Removal of the 5-chloro group of **130** was affected with palladium on carbon in a hydrogen atmosphere in methanol containing sodium acetate to give 7-*N,N*-diethylamino-3-phenylpyrazolo[1,5-*a*]pyrimidine (**131**).




Introduction of a substituted phenyl group into position 5 of pyrazolo[1,5-*a*]pyrimidin-7-one (**6**) was accomplished by the ring closure of 3-aminopyrazole with an appropriately substituted phenylacetoacetate in boiling glacial acetic acid as previously reported [57] from our laboratory for the synthesis of 5-phenylpyrazolo[1,5-*a*]pyrimidin-7-one (**61**). Application of this procedure gave 5-(*p*-chlorophenyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**63**) and 5-(*p*-nitrophenyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**64**).

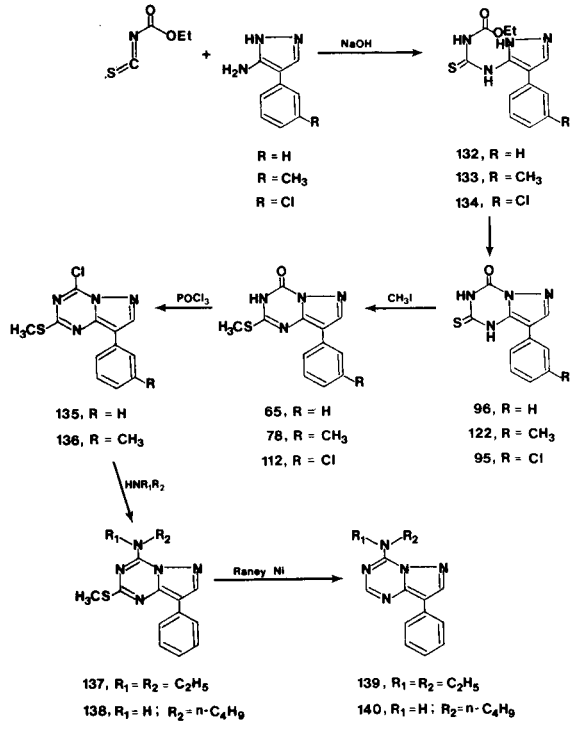
The synthesis of 5-chloropyrazolo[1,5-*a*]pyrimidin-7-one (**19**) was accomplished by treatment of 5,7-dichloropyrazolo[1,5-*a*]pyrimidine [58] with refluxing 5% aqueous sodium hydroxide.



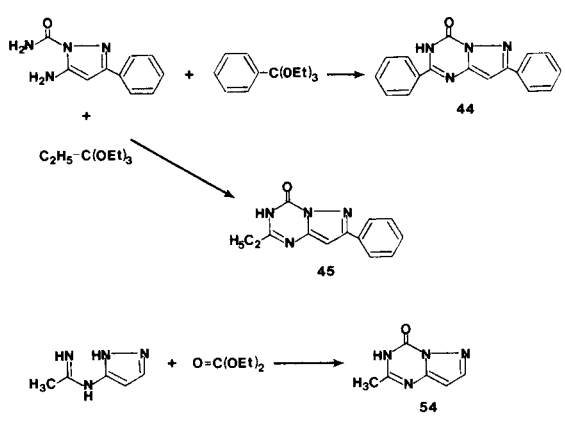




B. Pyrazolo[1,5-*a*]-*s*-triazines.

The synthesis of 8-phenyl-2-substituted pyrazolo[1,5-*a*]-*s*-triazines was accomplished from 3-amino-4-phenyl and substituted phenylpyrazoles by treatment with ethoxycarbonyl isothiocyanate to give the corresponding *N*-carbethoxy-*N*$^1$-(4-phenylpyrazol-3-yl)thioureas **132, 133** and **134**. Further treatment of these intermediates with 2*N* sodium hydroxide at room temperature resulted in ring closure to yield the corresponding 8-phenyl or substituted phenyl-2-thiopyrazolo[1,5-*a*]-*s*-triazin-4-one, **95, 96** and **122**, respectively. This type of ring closure has been reported [59] with 3-aminopyrazole and has been studied in our laboratory [13] as a route to various 4-substituted pyrazolo[1,5-*a*]-*s*-triazines. Methylation of the 2-thio function with methyl iodide in base gave the corresponding 8-phenyl-2-methylthiopyrazolo[1,5-*a*]-*s*-triazin-4-ones, **112, 65** and **78**, respectively. Treatment of 8-phenyl-2-methylthiopyrazolo[1,5-*a*]-*s*-triazin-4-one (**65**) and the corresponding 8-*m*-tolyl derivative (**78**) with refluxing phosphoryl chloride in the presence of *N,N*-diethylaniline gave the corresponding 4-chloro-8-phenyl- or 8-(*m*-tolyl)-2-methylthiopyrazolo[1,5-*a*]pyrimidine, **135** and **136**. Treatment of **135** with *N,N*-diethylamine resulted in the preparation of 8-phenyl-2-methylthio-4-*N,N*-diethylaminopyrazolo[1,5-*a*]-*s*-triazine (**137**). Similarly, *n*-butylamine and **135** gave a 78% yield of 4-*n*-butylamino-8-phenyl-2-methylthiopyrazolo[1,5-*a*]-*s*-triazine (**138**). Removal of the methylthio group of **137** and **138** with Raney Ni gave the corresponding 4-*N,N*-diethylamino- and 4-*n*-butylamino-8-phenylpyrazolo[1,5-*a*]-*s*-triazine, **139** and **140**, respectively.

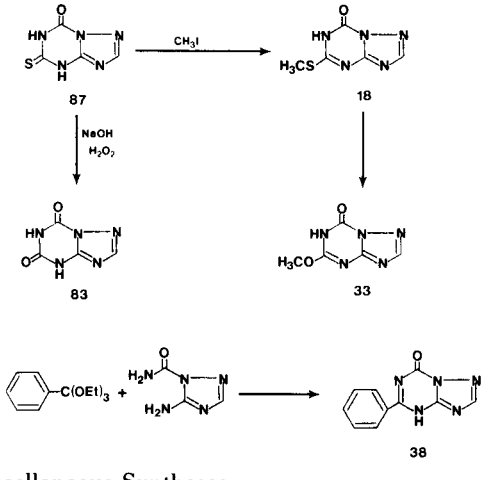



The synthesis of 2-ethyl-7-phenylpyrazolo[1,5-*a*]-*s*-triazin-4-one (**45**) was accomplished by ring closure of 3-amino-2-carbamoyl-5-phenylpyrazole [60] with refluxing triethyl orthopropionate to give a 73% yield of **45**. Similarly, ring closure of 3-amino-2-carbamoyl-5-phenylpyrazole [60] with triethyl orthobenzoate gave a 47% yield of 2,7-diphenylpyrazolo[1,5-*a*]-*s*-triazin-4-one, **44**. 2-Methylpyrazolo[1,5-*a*]-*s*-triazin-4-one (**54**) was prepared by ring closure of *N*-(pyrazol-3-yl)acetamidine [61] with diethylcarbonate in refluxing ethanol containing sodium ethoxide.



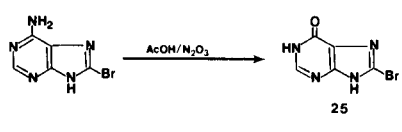
C. *s*-Triazolo[1,5-*a*]-*s*-triazines.

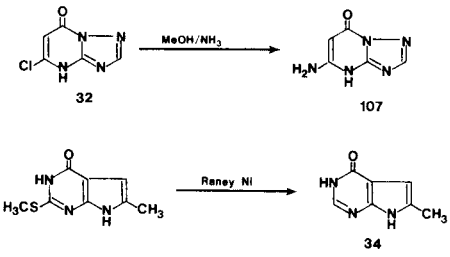
5-Methylthio-*s*-triazolo[1,5-*a*]-*s*-triazin-7-one (**18**) was prepared by methylation of 5-thio-*s*-triazolo[1,5-*a*]-*s*-triazin-7-one (**87**) [62], with methyl iodide in the presence of sodium hydroxide. Treatment of **18** with dry chlorine gas in methanol gave 5-methoxy-*s*-triazolo[1,5-*a*]-*s*-triazin-7-one (**33**) in 78% yield. The methyl sulfone or 5-chloro derivatives could be an intermediate in this reaction. Treatment of **87** with hydrogen peroxide gave *s*-triazolo[1,5-*a*]-*s*-triazin-5,7-dione (**83**, 5-azaxanthine) in 85% yield. 5-Phenyl-*s*-triazolo[1,5-*a*]-*s*-triazin-7-one (**38**) was prepared by ring closure of 3-amino-1,2,4-triazole-2-carboxamide [63] with triethyl orthobenzoate in *N,N*-dimethylformamide.



D. Miscellaneous Syntheses.

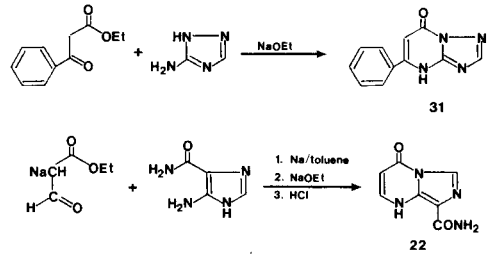
8-Bromohypoxanthine (**25**), was prepared in 61% yield by the treatment of 8-bromoadenine [64] with gaseous dinitrogen trioxide ($N_2O_3$) in 50% aqueous acetic acid. The guanine analog, 5-amino-*s*-triazolo[1,5-*a*]pyrimidin-7-one





(**107**), was prepared in 46.3% yield from 5-chloro-*s*-triazolo[1,5-*a*]pyrimidin-7-one (**32**) [65] by treatment with methanolic ammonia at 150° for 30 hours. 6-Methylpyrrolo-[2,3-*d*]pyrimidin-4-one (**34**) was prepared in 67% yield by Raney Ni dethiation of 6-methyl-2-methylthiopyrrolo-[2,3-*d*]pyrimidin-4-one [66]. 5-Phenyl-*s*-triazolo[1,5-*a*]pyrimidin-7-one (**31**) was prepared by ring closure of 3-amino-1,2,4-triazole with ethyl benzoylacetate in refluxing ethanol containing sodium ethoxide. The synthesis of 8-carboxamidoimidazo[1,5-*a*]pyrimidin-4-one (**22**) was achieved by ring closure of 4-aminoimidazole-5-carboxamide with ethyl formyl acetate (sodium salt) in refluxing ethanol.

A partial preliminary report fo the present study was presented at the 8th International Congress of Heterocyclic Chemistry, held at Graz, Austria in 1981 [100].



## EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (nmr) spectra were determined at 90 MHz with a JEOL FX 90Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. Ultraviolet spectra (uv, sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Robertson Labs, Florham Park, New Jersey. Evaporations were carried out under reduced pressure with the bath temperature below 30°.

3-(*m*-Tolyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**47**).

Method 1.

A suspension of sodium metal (4.0 g, 0.174 mole) in dry ether (1 liter) was stirred at room temperature while a mixture of ethyl formate (14.8 g, 0.1 mole) and ethyl acetate (17 g, 0.193 mole) was added dropwise. The resultant mixture was stirred at room temperature for 48 hours, at which time all of the sodium metal had reacted. The mixture was evaporated to dryness and the residue of crude ethyl α-formylacetate was dissolved in absolute ethanol (500 ml). This solution was stirred at room temperature while 3-amino-4-(*m*-tolyl)pyrazole (10.0 g, 0.0625 mole) [55] was added. The mixture was then heated at reflux for 4 hours and then evaporated to dryness. The solid residue was dissolved in 200 ml of water, treated with decolorizing carbon, and filtered. Acidification of the filtrate with concentrated hydrochloric acid afforded 8.3 g (59%) of 3-(*m*-tolyl)pyrazolo-

[1,5-*a*]pyrimidin-7-one (**47**), mp 308-310° dec.

Reprecipitation of this product from dilute sodium hydroxide solution did not change the melting point; uv: λ max (*p*H 1) 206 nm (ε 24,600), 272 (13,080); λ max (*p*H 11) 226 nm (ε 9,700), 327 (12,870); 293 sh.

*Anal.* Calcd. for $C_{13}H_{11}N_3O$: C, 69.50; H, 4.90; N, 18.70. Found: C, 69.50; H, 5.09; N 18.50.

Method 2.

A suspension of 6-carbethoxy-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**123**, 1.0 g) in 5 ml of 40% sulfuric acid was stirred and heated at reflux for 2 hours. At the end of this time, the solution was cooled and added to 10 ml of water. 2 *N* sodium hydroxide solution was added to this suspension until a *p*H of 4 was obtained. The white product was collected by filtration, washed with water, and then recrystallized from a mixture of dimethylformamide and water to afford analytically pure **47**, 0.5 g. This material was identical in all respects to the product obtained by Method 1.

6-Carbethoxy-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**123**).

A solution of 3-amino-4-(*m*-tolyl)pyrazole [55] (18.3 g, 0.1 mole) and diethyl ethoxymethylenemalonate (21.6 g, 0.1 mole) in 200 ml of acetic acid was stirred and heated at reflux for 3 hours. A complete solution was formed initially; however, after refluxing for about 1½ hours the product began to precipitate from the boiling solution. The mixture was allowed to cool to room temperature and the product was separated by filtration, washed with methanol, and dried. Recrystallization from a mixture of dimethylformamide and water afforded 18.6 g (63%) of analytically pure 6-carbethoxy-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**123**), mp 278-280° dec; uv: λ max (*p*H 1) 211 nm (ε 32,700), 292 (13,660); λ max (*p*H 11) 227 nm (ε 17,800), 319 (22,300).

*Anal.* Calcd. for $C_{16}H_{15}N_3O_3$: C, 64.60; H, 5.05; N, 14.12. Found; C, 64.53; H, 5.09; N, 14.11.

7-Chloro-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**124**).

To 6.75 g of 3-(*m*-tolyl)pyrimidin-7-one (**47**) was added 80 ml of phosphoryl chloride and 7 ml of *N,N*-dimethylaniline. The solution was refluxed for 2 hours and 60 ml of phosphoryl chloride was removed under vacuo and 150 ml of ice-water was added to the residue. The stirred mixture was kept cool and finally extracted with chloroform (3 × 150 ml). The combined organic layers were then washed with saturated aqueous sodium bicarbonate and finally dried over anhydrous sodium sulfate. Removal of the chloroform gave 6.0 g (82%) of product. Recrystallization from *n*-heptane gave light yellow needles, mp 132-133°.

*Anal.* Calcd. for $C_{13}H_{10}ClN_3$: C, 64.06; H, 4.14; N, 17.24. Found; C, 63,81; H, 4.33; N, 17.02.

7-Methoxy-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**49**).

To 1.22 g of **124** was added a solution of 0.23 g of sodium dissolved in 20 ml of anhydrous methanol. The solution was refluxed for 3 hours and the excess methanol evaporated. The residue was added to 50 ml of water and the crude product filtered, washed with water and recrystallized from ethanol to give 0.85 g (71%) of **49** as light yellow needles, 166.5-168°.

*Anal.* Calcd. for $C_{14}H_{13}N_3O$: C, 70.27; H, 5.48; N, 17.56. Found: C, 70.29; H, 5.67; N, 17.50.

7-Ethoxy-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**39**).

The synthesis of **39** was similarly accomplished from 1.22 g of **124**, which was treated with refluxing ethanol containing 0.23 g of sodium in 20 ml of absolute ethanol and worked up as with **49** to give 0.50 g (38%) of **39**, mp 138-140°.

*Anal.* Calcd. for $C_{15}H_{15}N_3O$: C, 66.90; H, 5.61; N, 15.61. Found: C, 66.98; H, 5.94; N, 15.93.

7-Benzyloxy-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**52**).

To 0.23 g of sodium dissolved in 5 ml of benzyl alcohol was added 20 ml of benzene and 1.22 g of **124**. The solution was refluxed for 2 hours and the excess solvents removed. The resulting oily residue was stirred with 30 ml of water and kept in the refrigerator overnight. The solid was collected by filtration and washed with cold water and then cold ethanol. The crude product was crystallized from ethanol to give 0.83 g (53%) of colorless needles of **52**, mp 138-140°.

*Anal.* Calcd. for $C_{20}H_{17}N_3O$: C, 76.15; H, 5.44; N, 13.32. Found: C, 76.29; H, 5.51; N, 13.53.

7-Ethylthio-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**50**).

To 0.68 g of ethanethiol in 30 ml of water containing 0.62 g of potassium hydroxide was added 0.85 g of **124**. The reaction mixture was stirred at room temperature for 18 hours and the crystals which formed were collected, washed with cold water and dried. The product was recrystallized from *n*-heptane:dimethylacetamide to give 0.5 g (53%) of **50** as yellow prisms, mp 136-138°.

*Anal.* Calcd. for $C_{15}H_{15}N_3S$: C, 66.87; H, 5.62; N, 15.60. Found: C, 66.69; H, 5.71; N, 15.92.

7-Ethylamino-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**125**).

To 1.22 g of **124** was added 10 ml of 70% aqueous ethylamine and 10 ml of ethanol. This reaction mixture was refluxed for 3 hours and then excess solvents removed. The residue was dissolved in 30 ml of 2*N* hydrochloric acid and 5% aqueous ammonia added until precipitation occurred. The precipitate was collected, washed with cold water and dried. The crude product was crystallized from *n*-heptane containing enough acetone to effect solution to give 0.5 g (40%) of **125** as pale yellow prisms, mp, 81-83°.

*Anal.* Calcd. for $C_{15}H_{16}N_4$: C, 71.40; H, 6.39; N, 22.21. Found: C, 71.49; H, 6.53; N, 22.16.

7-*N,N*-Dimethylamino-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**126**).

7-Chloro-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**124**, 1.22 g) was treated with 40% aqueous dimethylamine as for **125** and worked up and crystallized from *n*-heptane and acetone to give 0.95 g (75%) of **126** as yellow prisms, mp 107-109°.

*Anal.* Calcd. for $C_{15}H_{16}N_4$: C, 71.40; H, 6.39; N, 22.21. Found: C, 71.51; H, 6.50; N, 22.48.

7-*n*-Propylamino-3-(*m*-tolyl)pyrazolo[1,5-*a*]pyrimidine (**127**).

This compound was similarly prepared as for **125** except *n*-propylamine was employed to give a 36% yield of **127**, mp 101-102°.

*Anal.* Calcd. for $C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04. Found: C, 71.88; H, 7.06; N, 21.24.

5,7-Dichloro-3-phenylpyrazolo[1,5-*a*]pyrimidine (**128**).

To 30 g of 7-hydroxy-3-phenylpyrazolo[1,5-*a*]pyrimidin-5-one (**91**) [1] was added 300 ml of phosphoryl chloride and 30 ml of *N,N*-dimethylaniline. The solution was refluxed for 20 hours and the reaction mixture evaporated *in vacuo* using a steam bath as a source of heat. The residue was poured into 300 ml of ice-water and vigorously stirred and extracted with ethyl acetate (5 × 500 ml). The ethyl acetate solution was then washed with saturated sodium bicarbonate solution (5 × 200 ml) and dried over anhydrous sodium sulfate. Evaporation of ethyl acetate solution gave 28 g (80%) of crude product. Recrystallization of a small amount of the product from *n*-heptane gave an analytical sample of **128** as yellow needles, mp 154-156°.

*Anal.* Calcd. for $C_{12}H_7Cl_2N_3$: C, 54.56; H, 2.67; N, 15.91. Found: C, 54.63; H, 2.86; N, 15.86.

5-Chloro-7-ethylthio-3-phenylpyrazolo[1,5-*a*]pyrimidine (**129**).

To 1.32 g of **128** was added a solution of 0.93 g of ethanethiol and 0.84 g of potassium hydroxide dissolved in 30 ml of water. The solution was stirred at room temperature for 20 hours and the reaction mixture filtered and the solid washed with water. The dried solid was recrystallized from a mixture of *n*-heptane and acetone to give 0.94 (66%) of pure product, mp 143-145°.

*Anal.* Calcd. for $C_{14}H_{12}ClN_3S$: C, 58.01; H, 4.18; N, 14.50. Found: C, 58.21; H, 4.23; N, 14.55.

5-Chloro-3-phenylpyrazolo[1,5-*a*]pyrimidin-7-one (**67**).

To 5.28 g of **128** was added 50 ml of 5% sodium hydroxide. The solution was heated to reflux for 1.5 hours and then allowed to stand at room temperature overnight. The crystals of the sodium salt were collected and dissolved in 100 ml of boiling water. The hot solution boiled with decolorizing charcoal and filtered. The filtrate was acidified to a *p*H of 2 with dilute hydrochloric acid while hot. After the solution had cooled the product was collected by filtration and the product washed with cold water. The product was dried and recrystallized from ethanol to yield 2.5 g (51%) of **67**, mp 221-224°.

*Anal.* Calcd. for $C_{12}H_8ClN_3O$: C, 58.66; H, 3.29; N, 16.51. Found: C, 58.58; H, 3.48; N, 16.86.

5-Chloro-3-phenyl-7-*N,N*-diethylaminopyrazolo[1,5-*a*]pyrimidine (**130**).

To 1.32 g of **128** was added 0.73 g of *N,N*-diethylamine dissolved in 30 ml of absolute ethanol. The solution was refluxed for 6 hours and the solvent removed *in vacuo*. The residue was dissolved in 50 ml of water containing a small amount of dilute hydrochloric acid. This solution was neutralized with aqueous ammonia and the solid that separated was collected, washed with water and dried. Recrystallization from *n*-heptane gave 0.95 g (63%) of **130**, mp 68-70°.

*Anal.* Calcd. for $C_{16}H_{17}ClN_4$: C, 63.87; H, 5.70; N, 18.62. Found: C, 63.61; H, 5.81; N, 18.73.

7-Diethylamino-3-phenylpyrazolo[1,5-*a*]pyrimidine (**131**).

To 0.9 g of **130** was added 100 ml of methanol containing 0.25 g of sodium acetate and 0.3 g of 5% Pd/C catalyst. This solution was placed on a hydrogenator and shaken for 5 hours at two atmospheres pressure of hydrogen. The solution was then filtered through a Celite pad and the filtrate evaporated to dryness. The residue was triturated with water and the residue was collected by filtration and dried. The crude product was crystallized from *n*-heptane to yield 0.4 g of **131**, mp 75°.

*Anal.* Calcd. for $C_{16}H_{18}N_4$: C, 72.15; H, 6.81; N, 21.04. Found: C, 72.04; H, 6.94; N, 20.95.

Synthesis of *N*-Carbethoxy-*N*$^1$-(4-phenyl)- and (4-Substitutedphenyl)pyrazol-3-yl-thioureas, (**132**, **133** and **134**).

3-Amino-4-phenylpyrazole (5 g) [55] was dissolved in 30 ml of boiling ethyl acetate. The solution was cooled to 5° and 4.12 g of carbonylisothiocyanate in 60 ml of ethyl acetate was added over a three-minute period and the solution was stirred at room temperature overnight. The solution was filtered and the product washed with ethyl acetate to yield 3.7 g (40%) of **132**. Recrystallization of a small sample from ethyl acetate gave a pure product, mp 203-205°.

*Anal.* Calcd. for $C_{13}H_{14}N_4O_2S$: C, 53.45; H, 5.48; N, 19.17. Found: C, 53.60; H, 5.26; N, 19.26.

*N*-Carbethoxy-*N*$^1$-(4-*m*-tolylpyrazol-3-yl)thiourea (**133**).

This compound was similarly prepared in 44% yield. Recrystallization from water-ethanol gave an analytical sample, mp 179-181°.

*Anal.* Calcd. for $C_{14}H_{16}N_4O_2S$: C, 55.30; H, 5.26; N, 18.42. Found: C, 55.33; H, 5.48; N, 18.61.

*N*-Carbethoxy-*N*$^1$-(4-*m*-chlorophenylpyrazol-3-yl)thiourea (**134**).

This compound was similarly prepared in 55% yield. The crude material was recrystallized from water-methanol to give a pure sample of **134**, mp 261-263°.

*Anal.* Calcd. for $C_{13}H_{12}ClN_4O_2S$: C, 48.25; H, 3.71; N, 17.11. Found: C, 48.31; H, 3.73; N, 16.99.

Synthesis of 8-Phenyl- and 8-Substitutedphenyl-2-thiopyrazolo[1,5-*a*]-*s*-triazin-4-ones, (**95**, **96** and **122**).

To 15.6 g of *N*-carbethoxy-*N*$^1$-(4-phenylpyrazol-3-yl)thiourea (**132**) was added 250 ml of 2*N* sodium hydroxide. The solution was stirred overnight at room temperature and acidified with acetic acid to yield 12.5 g (92%) of crude **96**. A small sample was purified for analysis by recrystallization from aqueous *N,N*-dimethylformamide to give **96**, mp 251-253°.

*Anal.* Calcd. for $C_{11}H_8N_4OS$: C, 54.10; H, 3.28; N, 22.95. Found: C, 54.08; H, 3.27; N, 23.27.

8-(*m*-Chlorophenyl)-2-thiopyrazolo[1,5-*a*]-*s*-triazin-4-one (**96**).

This compound was similarly prepared from **134** to give a 94% yield, mp 294-296°.

*Anal.* Calcd. for $C_{11}H_7ClN_4OS$: C, 47.40; H, 2.52; N, 20.10. Found: C, 47.40; H, 2.52; N, 20.03.

8-(*m*-Tolyl)-2-thiopyrazolo[1,5-*a*]-*s*-triazin-4-one (**122**).

This compound was similarly prepared from **133** in 97% yield, mp 234-236°.

*Anal.* Calcd. for $C_{12}H_{10}N_4OS \cdot \frac{1}{2}H_2O$: C, 53.95; H, 4.12; N, 20.97. Found: C, 53.90; H, 4.27; N, 21.01.

Synthesis of 8-Phenyl- and 8-Substitutedphenyl-2-methylthiopyrazolo-[1,5-*a*]-*s*-triazin-4-ones (**65**, **78** and **112**).

To a solution of **122** (8.8 g) in ethanol (120 ml) was added a solution of 2.7 g of sodium hydroxide in 50 ml of water, followed by 2.13 ml of methyl iodide and the mixture was stirred for 2 hours. The product was collected by filtration, washed with ethanol and dried to yield 9.3 g of **78**. A small sample was recrystallized from water-ethanol for analysis, mp 276-278°.

*Anal.* Calcd. for $C_{13}H_{12}N_4OS$: C, 57.40; H, 4.41; N, 20.57. Found: C, 57.57; H, 4.52; N, 20.57.

The methylation of **96** similarly gave **65** in 71% yield; mp, after recrystallization from aqueous *N,N*-dimethylformamide, 325-327°.

*Anal.* Calcd. for $C_{12}H_{10}N_4OS$: C, 55.80; H, 3.88; N, 21.70. Found: C, 56.05; H, 3.82; N, 21.49.

Methylation of **95** with methyl iodide similarly gave **112** in 92% yield. Recrystallization from water-ethanol gave pure product, mp 274-276°.

*Anal.* Calcd. for $C_{12}H_9ClN_4OS$: C, 49.25; H, 3.07; N, 19.14. Found: C, 49.41; H, 3.29; N, 19.02.

2-Methylthio-4-chloro-8-phenylpyrazolo[1,5-*a*]-*s*-triazine (**135**).

To 13.5 g of **65** was added 150 ml of phosphoryl chloride and 14 ml of *N,N*-diethylaniline (mono-free). The solution was refluxed for 3 hours and the excess phosphoryl chloride removed under *vacuo* using a steam bath as a source of heat. The residue was poured onto 250 ml of ice-water and the solution stirred vigorously for 15 minutes. The solution was extracted with 5 × 200 ml of chloroform, the chloroform solution washed with saturated sodium bicarbonate solution (4 × 200 ml) and dried over anhydrous sodium sulfate. Careful removal of the chloroform gave 12.4 g (86%) of **135**, which was used directly for nucleophilic substitution. A small sample was purified by sublimation for analysis, mp 141-143°.

*Anal.* Calcd. for $C_{12}H_9ClN_4S$: C, 52.10; H, 3.25; N, 20.25. Found: C, 52.10; H, 3.45; N, 20.31.

2-Methylthio-4-chloro-8-(*m*-tolyl)pyrazolo[1,5-*a*]-*s*-triazine (**136**).

To 19.0 g of **78** in 175 ml of phosphoryl chloride was added 20 ml of *N,N*-dimethylaniline and the solution refluxed for 1 hour. After removal of the excess phosphoryl chloride under *vacuo*, the residue was poured onto 300 ml of ice-water and stirred vigorously. The solution was then extracted with ether (4 × 200 ml) and the ethereal solution washed with aqueous saturated sodium bicarbonate solution and dried over anhydrous sodium sulfate. Evaporation of the ether gave 17.6 g (87%) of **136**. A small sample was purified by sublimation for analysis, mp 129-131°.

*Anal.* Calcd. for $C_{13}H_{11}ClN_4S$: C, 53.70; H, 3.79; N, 19.30. Found: C, 54.01; H, 3.87; N, 19.03.

2-Methylthio-4-*N,N*-diethylamino-8-phenylpyrazolo[1,5-*a*]-*s*-triazine (**137**).

To 6.2 g of **135** was added 60 ml of ethanol and 5 ml of diethylamine. The solution was stirred at room temperature for 1 hour and allowed to evaporate overnight to dryness in the hood. The residue was recrystalliz-

ed from a water-ethanol mixture to yield 5.3 g (76%) of **137**, mp 107-109°.

*Anal.* Calcd. for $C_{16}H_{19}N_5S$: C, 61.40; H, 6.07; N, 22.33. Found: C, 61.60; H, 6.08; N, 22.32.

2-Methylthio-4-*n*-butylamino-8-phenylpyrazolo[1,5-*a*]-*s*-triazine (**138**).

In a similar manner as for **137**, reaction of **135** and *n*-butylamine gave 5.5 g (78%) of **138**, mp 89-91°.

*Anal.* Calcd. for $C_{16}H_{19}N_5S$: C, 61.40; H, 6.07; N, 22.33. Found: C, 61.55; H, 6.10; N, 22.30.

4-*N,N*-Diethylamino-8-phenylpyrazolo[1,5-*a*]-*s*-triazine (**139**).

To 3.2 g of **137** was added 125 ml of absolute ethanol and 16 g of wet Raney nickel previously washed repeatedly to remove excess sodium hydroxide and stored over ethanol. To this reaction mixture was added 5 ml of concentrated aqueous ammonia and the solution was refluxed for 2 hours. The solution was filtered hot and the excess solvent removed to give a semisolid which was recrystallized from aqueous ethanol to give 1.7 g (62%) of **139**, mp 90-91°.

*Anal.* Calcd. for $C_{15}H_{17}N_5$: C, 67.40; H, 6.37; N, 26.20. Found: C, 67.30; H, 6.42; N, 26.31.

4-*n*-Butylamino-8-phenylpyrazolo[1,5-*a*]-*s*-triazine (**140**).

Three and three-tenths g of **138** was treated with Raney nickel as for **137** to give 1.7 g (60%) of **140**, mp 78-80°.

*Anal.* Calcd. for $C_{15}H_{17}N_5$: C, 67.40; H, 6.37; N, 26.20. Found: C, 67.60; H, 6.60; N, 26.20.

8-Bromohypoxanthine (**25**).

8-Bromoadenine (1.0 g) [64] was suspended in 50% aqueous acetic acid (250 ml) and gaseous dinitrogen trioxide ($N_2O_3$) was passed through the solution at room temperature for 1 hour. At this time 8-bromoadenine and all dissolved and the solution attained a blue color. Evaporation of the solution gave a white solid which was collected by filtration and crystallized from water to give 0.61 g (61%) of a pale yellow crystalline solid, mp >300°.

*Anal.* Calcd. for $C_5H_3BrN_4O\cdot\frac{1}{2}H_2O$: C, 26.80; H, 1.79; N, 25.01; Br, 35.67. Found: C, 27.15; H, 2.04; N, 25.20; Br, 35.46.

5-Phenyl-1,2,4-triazolo[1,5-*a*]-*s*-triazin-7-one (**38**).

To 2.54 g of 3-amino-1,2,4-triazole-2-carboxamide [63] in 20 ml of *N,N*-dimethylformamide was added 4.5 g of triethyl orthobenzoate and 1.5 ml of glacial acetic acid. The solution was refluxed for 72 hours. The solvents were removed to give an orange viscous oil. Trituration of this oil with ethyl ether and a small amount of methanol gave 1.4 g of ivory solid, which was purified by reprecipitation from hot dilute aqueous ammonia with acetic acid, mp 220-222°.

*Anal.* Calcd. for $C_{10}H_7N_5O$: C, 56.28; H, 3.28; N, 32.83. Found: C, 56.33; H, 3.31; N, 32.85.

2,5-Diphenylpyrazolo[1,5-*a*]-*s*-triazin-7-one (**44**).

A mixture of 2.02 g (10 mmoles) of 3-amino-2-carbamoyl-5-phenylpyrazole [67] and 2.68 g (12 mmoles) of triethyl orthobenzoate in 20 ml of dimethylformamide was refluxed for 24 hours. The reaction mixture was evaporated to dryness *in vacuo*, and ethanol was added to the resulting residue. The insoluble solid was collected by filtration, washed with ethanol and dried to give 1.35 g (47%) of product. Recrystallization from aqueous dimethylformamide gave analytically pure **44**, mp 297-300°.

*Anal.* Calcd. for $C_{17}H_{12}N_4O$: C, 70.82; H, 4.20; N, 19.44. Found: C, 70.75; H, 3.96; N, 19.51.

5-Ethyl-2-phenylpyrazolo[1,5-*a*]-*s*-triazin-7-one (**45**).

A mixture of 2.02 g (10 mmoles) of 3-amino-2-carbamoyl-5-phenylpyrazole [67] and 5 ml of triethyl orthopropionate was refluxed for 24 hours. After standing overnight at room temperature, the precipitated crystals were collected by filtration, washed with ether and dried to give 1.75 g (73%) of colorless crystals. Recrystallization from aqueous dimethylsulfoxide gave analytically pure 5-ethyl-2-phenylpyrazolo[1,5-*a*]-*s*-triazin-7-one **45**), mp 244-245°.

*Anal.* Calcd. for $C_{13}H_{12}N_4O$: C, 64.98; H, 5.03; N, 23.32. Found: C, 64.98; H, 4.99; N, 23.19.

2-Methylpyrazolo[1,5-*a*]-*s*-triazin-4-one (**54**).

*N*-(Pyrazol-3-yl)acetamidine acetate (4.6 g) [61] was added to 100 ml of ethanol containing 5.75 g of dissolved sodium. To this solution was added 25 ml of diethylcarbonate and the solution was refluxed for 5 hours. The solution was then evaporated to dryness and 50 ml of water added to the residue and the *p*H of the solution adjusted to 4 with acetic acid. Evaporation of this solution to dryness gave a residue that was triturated with 50 ml of cold water to give crystals which were filtered and washed with a small amount of ice water, followed by acetone. The product was recrystallized from ethanol to give 1.45 g (39%) of colorless needles, mp 270-272°.

*Anal.* Calcd. for $C_6H_6N_4O$: C, 47.98; H, 4.03; N 37.32. Found: C, 47.80; H,4 .29; N, 37.11.

5-Phenyl-*s*-triazolo[1,5-*a*]pyrimidin-7-one (**31**).

To 8.4 g of 3-amino-1,2,4-triazole and 22 g of ethyl benzoyl acetate in 150 ml of ethanol was added a solution of 2.7 g of sodium dissolved in 150 ml of ethanol and the mixture was refluxed for 4 hours. The solution was neutralized with acetic acid and allowed to stand in the refrigerator overnight. The product was collected by filtration and recrystallized from aqueous *N,N*-dimethylformamide to give 8.2 g of **31**, mp 291-292°.

*Anal.* Calcd. for $C_{11}H_8N_4O$: C, 61.80; H, 3.77; N, 26.40. Found: C, 61.78; H, 3.83; N, 26.65.

5-(*p*-Chlorophenyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**63**).

To 4.0 g of 3-aminopyrazole was added 11.0 g of *p*-chlorobenzoyl chloride and ethyl acetoacetate according to the general directions for the synthesis of ethyl benzoylacetate [68]. The mixture was heated at 120° for 10 minutes. After about 5 minutes the reaction mixture solidified. The solid was dissolved in warm dilute sodium hydroxide, treated with charcoal and filtered through Celite. The hot filtrate was acidified with acetic acid and the resulting white solid was collected, washed, with water, dried and recrystallized from aqueous *N,N*-dimethylformamide to give 5.7 g of **63** as needles, mp 308-310°.

*Anal.* Calcd. for $C_{12}H_5ClN_3O$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.90; H, 3.38; N, 16.82.

5-(*p*-Nitrophenyl)pyrazolo[1,5-*a*]pyrimidin-7-one (**64**).

This compound was similarly prepared from 3-aminopyrazole and *p*-nitrobenzoylacetate.

5-Amino-*s*-triazolo[1,5-*a*]pyrimidin-7-one (**107**).

5-Chloro-*s*-triazolo[1,5-*a*]pyrimidin-7-one (**32**, 5.2 g, 30 mmoles) [65] was dissolved in methanolic ammonia (150 ml, saturated at 0°) and the solution was heated in a steel reaction vessel at 150° for 30 hours. After cooling, the reaction mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in a minimum of water, adsorbed onto silica gel (~20 g) and the excess solvent was evaporated. Co-evaporation from the solid mass with toluene (5 × 50 ml) gave dry residue, which was loaded onto a silica gel column (4 × 60 cm) packed with ethyl acetate. The column was eluted with ethyl acetate:water:1-propanol (4:2:1, upper phase). The appropriate, homogeneous fractions, were combined, evaporated to dryness and the residue was crystallized from aqueous ethanol to yield 2.1 g (46.3%) of **107**, mp >315° dec; ir (potassium bromide): 1665 (>C=O), and 3320 (-$NH_2$) $cm^{-1}$; uv: λ max (*p*H 1) 239 nm (ε 3,300), 278 (6,600); λ max (*p*H 7 and 11), 254 nm (ε 4,100), 281 (7,100).

*Anal.* Calcd. for $C_5H_5N_5O\cdot H_2O$: C, 35.50; H, 4.17; N, 41.40. Found: C, 35.26; H, 4.43; N, 41.68.

5-Methylthio-*s*-triazolo[1,5-*a*]-*s*-triazin-7-one (**18**).

A solution of 5-thio-*s*-triazolo[1,5-*a*]-*s*-triazin-7-one [62] (**87**, 5.9 g, 35 mmoles) in 150 ml of ethanol containing 1.75 *N* sodium hydroxide (50 ml)

was stirred at room temperature, while methyl iodide (5 g) was added dropwise. Ten minutes after the addition was complete, the white sodium salt of the product began to separate. The mixture was stirred for an additional 20 minutes and the sodium salt was collected by filtration. The salt was dissolved in minimum volume of water and acidified (*p*H 2) with 10% sulfuric acid. The precipitate that separated was collected, washed with cold water (2 × 10 ml) and crystallized from aqueous ethanol as needles to yield 5.2 g (81.2%) of **18**, mp 303° dec; ir (potassium bromide): 1365 (-$SCH_3$) and 1770 (>C=O) $cm^{-1}$; uv: λ max (*p*H 1) 228 sh, nm (ε 5,500), 273 (14,600); λ max (*p*H 7 and 11) 235 nm (ε 18,000), 268 (13,300).

*Anal.* Calcd. for $C_5H_5N_5OS$: C, 32.79; H, 2.75; N, 38.25. Found: C, 32.89; H, 2.71; N, 38.37.

*s*-Triazolo[1,5-*a*]-*s*-triazine-5,7(4*H*,6*H*)-dione (**83**).

A solution of 5-thio-*s*-triazolo[1,5-*a*]-*s*-triazine-5,7(4*H*,6*H*)-dione (**87**, 3.38 g, 20 mmoles) [62] in 160 ml of 0.25 *N* sodium hydroxide was cooled to 0°. With good stirring 30% hydrogen peroxide (40 ml) was added dropwise, keeping the temperature between 0-5°. After the addition was complete, the mixture was stirred for an additional 15 minutes and allowed to warm to room temperature before it was acidified (*p*H 1) with 2 *N* sulfuric acid. The acidic mixture was chilled overnight, the precipitate that separated was collected by filtration, and washed with cold water (2 × 25 ml). Crystallization from water gave an analytical sample to yield 2.6 g (85%) of **83**, mp >320° dec.

*Anal.* Calcd. for $C_4H_3N_5O_2$: C, 31.38; H, 1.98; N, 45.75. Found: C, 31.02; H, 1.87; N, 45.93.

5-Methoxy-*s*-triazolo[1,5-*a*]-*s*-triazin-7-one (**33**).

5-Methylthio-*s*-triazolo[1,5-*a*]-*s*-triazin-7-one (**18**, 5.5 g, 30 mmoles) was suspended in anhydrous methanol (100 ml) and dry chlorine gas was passed into the cooled mixture for 30 minutes with stirring, at such a rate that the reaction temperature remained at <10°, with external cooling. The clear pale yellow solution was evaporated in a stream of dry air to approximately 20 ml. The white precipitate which had formed was collected, washed with cold water (2 × 10 ml) followed by ethanol (10 ml). The residue was crystallized from aqueous ethanol as needles, to yield 3.9 g (78%) of **33**, mp 212°; ir (potassium bromide): 1140, 2860 (-$OCH_3$) and 1780 (>C=O) $cm^{-1}$; uv: λ max (*p*H 1) 239 nm (ε 8,700); λ max (*p*H 7 and 11) 239 nm (ε 9,700).

*Anal.* Calcd. for $C_5H_5N_5O_2$: C, 35.93; H, 3.02; N, 41.91. Found: C, 35.68; H, 2.90; N, 42.14.

5-Chloropyrazolo[1,5-*a*]pyrimidin-7-one (**19**).

A solution of 5,7-dichloropyrazolo[1,5-*a*]pyrimidine [58] (1.88 g, 10 mmoles) in 5% aqueous sodium hydroxide (20 ml) was heated under reflux for 30 minutes. The mixture was cooled to 0° and the white solid that precipitated was collected by filtration, dissolved in water (25 ml) and acidified (*p*H 2) with 10% hydrochloric acid. The solid was collected, washed with cold water (2 × 10 mmoles) and crystallized from aqueous ethanol to yield 1.3 g (77%) of **19**, mp 298-300° dec; ir (potassium bromide): 780 (C-Cl), 1690 (>C=O) $cm^{-1}$; uv: λ max (*p*H 1) 256 nm (ε 2,050), 295 (2,050); λ max (*p*H 7), 276 nm (ε 3,200), 295 sh (2,600); λ max (*p*H 11) 276 nm (ε 3,900), 295 sh (3,200).

*Anal.* Calcd. for $C_6H_4ClN_3O$: C, 42.50; H, 2.37; N, 24.78; Cl, 20.90. Found: C, 42.31; H, 2.36; N, 24.93; Cl, 20.52.

6-Methylpyrrolo[2,3-*d*]pyrimidin-4-one (**34**).

To a suspension of 2-methylthio-6-methylpyrrolo[2,3-*d*]pyrimidin-4-one [66] (10.35 g, 50 mmoles) in water (250 ml) containing concentrated ammonium hydroxide (60 ml) was added Raney nickel (45 g, wet weight), and the mixture was heated at reflux for 4 hours. An additional charge of Raney nickel (10 g) was added and refluxing was continued for a total of 6 hours. The mixture was filtered while hot through a Celite pad, and the filter cake was washed with hot water (2 × 25 ml). The combined filtrates were concentrated to about 75 ml, cooled and the white precipitate that separated was collected by filtration. Crystallization from a large volume of water gave analytically pure material, yield 5.0 g (67%) of **34**, mp 230-231° dec; ir (potassium bromide): 1650 (>C=O) $cm^{-1}$; uv: λ max (*p*H 1) 268 nm (ε 3,600); λ max (*p*H 7), 267 nm (ε 4,400); λ max (*p*H 11) 268 nm (ε 3,100).

*Anal.* Calcd. for $C_7H_7N_3O$: C, 56.37; H, 4.73; N, 28.17. Found; C, 36.38; H, 4.71; N, 28.05.

8-Carboxamidoimidazo[1,5-*a*]pyrimidin-3-one (**22**).

Sodium metal (9.2 g), in 300 ml of toluene was heated to reflux and then rapidly cooled with stirring to produce sodium sand. When the toluene had cooled to room temperature, 29 g of ethyl formate and 35.3 g of ethyl acetate were mixed and slowly added with stirring at such a rate that the inside temperature was maintained below 25°. After the addition, the solution was stirred for an additional 15 hours. Then 4.6 g of sodium was added to 300 ml of absolute ethanol and 32.5 g of 4-aminoimidazole-5-carboxamide hydrochloride was added to the sodium ethoxide solution. The ethanolic solution was then slowly added to the sodium formylacetate solution and the solution kept at room temperature for 1 hour and finally refluxed for 5 hours. The excess solvents were removed under reduced pressure and the residue dissolved in 500 ml of water and separated from the residual toluene. The solution was neutralized with approximately 100 ml of glacial acetic acid to precipitate the product, which was filtered, washed and dried to give 6.4 g of crude **22**. Reprecipitatian of the product from hot dilute aqueous ammonia with acetic acid gave a pure product, 5.2 g.

*Anal.* Calcd. for $C_7H_6N_4O_2$: C, 47.15; H, 3.37; N, 31.43. Found: C, 47.19; H, 3.39; N, 31.45.

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