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(54) **1H-Pyrimido-(4,5-c)-1,2-diazepines, methods for their preparation and compositions containing them; their use as intermediates for heterocyclic transformations.**

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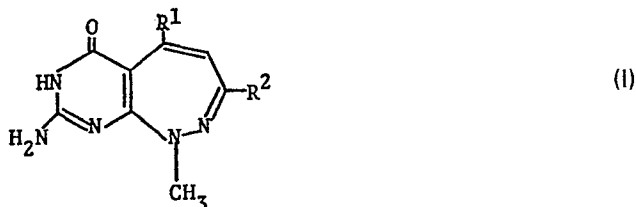
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1H-Pyrimido-(4,5-c)-1,2-diazepines, methods for their preparation and compositions containing them; their use as intermediates for heterocyclic transformations

This invention relates to 1H-pyrimido[4,5-c]-1,2-diazepines, methods of their preparation, compositions and formulations containing them and their uses as antimicrobial agents, and as intermediates for heterocyclic transformations.

The present invention provides novel 1H-pyrimido[4,5-c]-1,2-diazepines of formula (I), or their tautomers, or salts thereof,



wherein R¹ and R² are the same or different and are selected from a lower alkyl group, a phenyl group (optionally substituted by one or more hydroxy or lower alkoxy groups), a pyridyl group or a group CO₂R in which R is a lower alkyl group; provided that when R¹ is a group CO₂R then R² may only be a lower alkyl group.

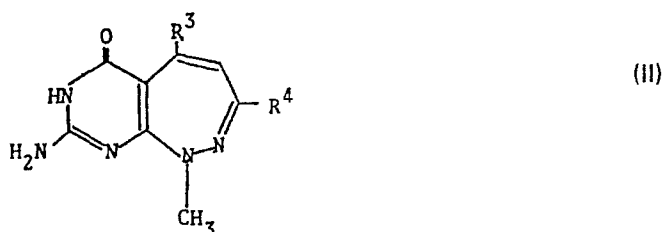
The term "lower" as used herein in conjunction with an alkyl or alkoxy group is indicative of the fact that such groups have from 1 to 4 carbon atoms arranged in a straight or branched chain.

It is to be understood that compounds where tautomerism is possible between, on the one hand, a hydroxy group and an oxo group, and on the other hand, an amino group and an imino group, at a particular position on the (1H)-pyrimido[4,5-c]-1,2-diazepines of formula (I), the more stable forms are respectively, the oxo group and the amino group. The formulae used in the present specification show the more stable form of such compounds.

It should be noted that the compounds of formula (I) possess a completely new type of skeletal ring structure, that is, the pyrimido[4,5-c]-1,2-diazepine bicyclic rings. Accordingly, an important advantage of the present invention is that a whole new area of novel chemistry has been opened up for further investigation by the provision herein of processes enabling the production of compounds of formula (I).

The above compounds of formula (I) have been found to be particularly susceptible to ring-opening and ring-closure rearrangement reactions. Depending upon the nature of the substituents, their location, and reaction conditions, the compounds of the present invention may be converted to either pyrido[2,3-d]pyrimidines, pyrimido[4,5-c]pyridazines, or pyrazolo[2,3-d]pyrimidines which compounds may then be converted by reactions well known in the art, for example hydrolysis, halogenation, amination, reduction, oxidation, nucleophilic substitution, electrophilic substitution and elimination, to compounds with the same basic skeletal ring structure but different substituents which compounds have biological activity. For example the pyrido[2,3-d]-pyrimidines of U.K. Patent Specification Nos. 774 094 and 774 095 have activity against lactic acid bacteria, the pyrido[2,3-d]pyrimidines of U.K. Patent Specification No. 1 129 084 are useful as diuretics, the pyrido[2,3-d]pyrimidines of U.K. Patent Specification No. 1 427 508 are active against micro-organisms which utilise the *de novo* synthesis of riboflavin, the pyrazolo[2,3-d]pyrimidines of U.K. Patent Specification No. 798 646 are active as antimetabolites in purine synthesis, and the pyrimido[4,5-c]pyridazines of copending European Patent Application No. 383 have activity as inhibitors of the enzyme dihydropteroate synthetase which enables micro-organisms to synthesise an essential intermediate in the production of tetrahydrofolate co-factors. The compounds of the present invention may therefore be used as intermediates for the production of pharmaceuticals which have one of the above three specified ring structures.

Within the class of (1H)-pyrimido[4,5-c]-1,2-diazepines of formula (I) there is a group of compounds represented by formula (II):



wherein R³ is a phenyl group optionally substituted with one or more hydroxy groups; and R⁴ is a group

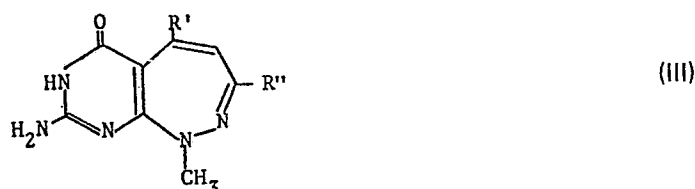
CO₂R in which R is as hereinbefore defined, which exhibit dihydropteroic acid biosynthesis inhibitory activity.

Thus, the above compounds of formula (II) inhibit one of the enzymes, namely dihydropteroate synthetase, which enables micro-organisms to synthesise an essential intermediate in the production of tetrahydrofolate co-factors. Most of these co-factors are one-carbon adducts of tetrahydrofolic acid and they are essential metabolites in cells of the biosynthesis of purines, thymidylic acid, serine and several other biologically important compounds. Man and other higher animals are unable to synthesise folic acid and therefore they have to obtain it from food which contains the required preformed folates.

On the other hand, most micro-organisms synthesise folic acid from simpler chemicals. Generally the biosynthetic process first provides 'dihydropteridine' (Pt), via 2 - amino - 4 - hydroxy - 6 - hydroxymethyl - 7,8 - dihydropteridine (HMPt) pyrophosphate ester itself obtained from its immediate precursor HMPt in the presence of the enzyme hydroxymethyldihydropteridine pyrophosphokinase (HMPPS). Pt then condenses with p-aminobenzoic acid (pAB) in the presence of the enzyme dihydropteroate synthetase to form dihydropteric acid (DPtA). This intermediate further condenses with a glutamate to form dihydrofolic acid (DFA or 'folate') which is then enzymatically reduced to produce the essential tetrahydrofolate.

As examples of compounds which are particularly active and fall within this class are 8 - amino - 3 - carboxy - 1 - methyl - 5 - phenyl - 1H - pyrimido[4,5 - c] - 1,2 - diazepin - 6(7H) - one and 8 - amino - 3 - carbomethoxy - 1 - methyl - 5 - (3 - hydroxyphenyl) - 1H - pyrimido[4,5 - c] - 1,2 - diazepin - 6(7H) - one. These compounds additionally have the ability to potentiate the antimicrobial activity of a 2,4-diamino-5-benzyl pyrimidine and/or a sulphonamide, for example trimethoprim or sulphamethoxazole.

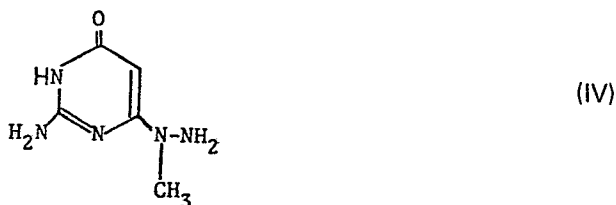
Also within the class of (1H)-pyrimido[4,5-c]-1,2-diazepines of formula (III):



wherein R' is a phenyl group optionally substituted with one or more lower alkoxy groups and R'' is a group —CO₂R in which R is as hereinbefore defined, or a phenyl group which have anti-protozoal, for example anti-coccidial, activity.

As examples of compounds falling within formula (III) and of particular interest are 8 - amino - 3 - carbomethoxy - 1 - methyl - 5 - (3,4,5 - trimethoxyphenyl) - 1H - pyrimido[4,5 - c] - 1,2 - diazepin - 6(7H) - one; 8 - amino - 3 - carboxy - 1 - methyl - 5 - (2,4 - dimethoxyphenyl) - 1H - pyrimido[4,5 - c] - 1,2 - diazepin - 6(7H) - one and 8 - amino - 3,5 - diphenyl - 1 - methyl - 1H - pyrimido[4,5 - c] - 1,2 - diazepin - 6(7H) - one. Such compounds alternatively have the ability to potentiate the antimicrobial activity of combinations of a 2,4-diamino-5-benzylpyrimidine and a sulphonamide. Thus compounds of formula (III) may for example, potentiate the antiprotozoal activity of a mixture of diaveridine and sulphaquinoxaline.

The compounds of formula (I) may be prepared by the reaction of 6 - (1 - methylhydrazino)isocytosine, represented by formula (IV):



55 with an α,γ -dicarbonyl compound of formula (V):



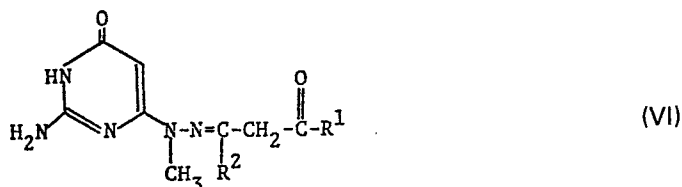
wherein R¹ and R² are as hereinbefore defined.

The preparation is suitably achieved by refluxing in a suitable solvent, most desirably a hydroxylic solvent such as a C₁—C₄ alkanol, glacial acetic and/or water, at a reflux temperature for up to several days. Optimally the reaction is carried out in refluxing methanol.

65 In the preparation of compounds of formula (I) some other bicyclic compound may be formed as a

by-product. In such instances it may be necessary to isolate the required compound by the usual procedures known in the art.

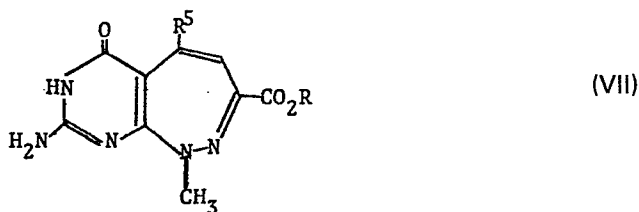
The reaction of the compound of formula (IV) with those of formula (V) is a two-step process, the first step being a condensation reaction and the second step a cyclisation of the so formed intermediate. It is believed that the intermediate formed has the formula (VI):



15 wherein R¹ and R² are as hereinbefore defined. The compounds of formula (VI) are novel compounds which form a further aspect of the invention. It is to be understood that the compounds of formula (VI) may either be converted *in situ* to compounds of formula (I) by continuing the reaction to completion under the original conditions or alternatively may be isolated and then converted to compounds of formula (I) under the reaction conditions suitable for the direct conversion of the intermediate to
20 compounds of formula (I).

The type of dicarbonyl compound of formula (V) used in the preparation of compounds of formula (I) will of course depend upon the substituents R¹ and R² in the compounds of formula (I).

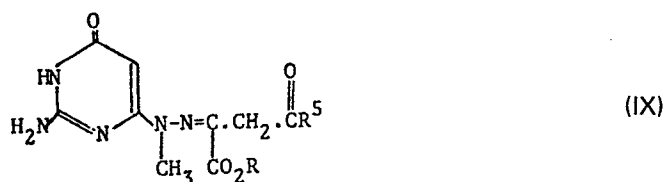
Thus when R¹ is either a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group and R² is a group CO₂R in which R is a lower alkyl group, viz a
25 compound of formula (VII):



35 where R is as defined above and R⁵ is a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group, the dicarbonyl compound will be an α,γ -diketoester of formula (VIII):

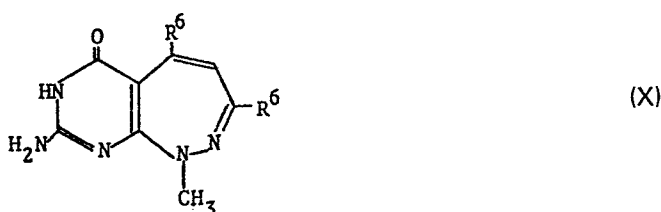


where R and R⁵ are as defined hereinabove. In this case the believed intermediate is a compound of formula (IX):



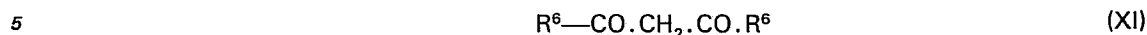
where R and R⁵ are as defined hereinabove. Compounds of formula (IX) are novel and form a further aspect of the invention.

55 When, in the compounds of formula (I), R¹ and R² are the same and are both a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group, viz a compound of formula (X):

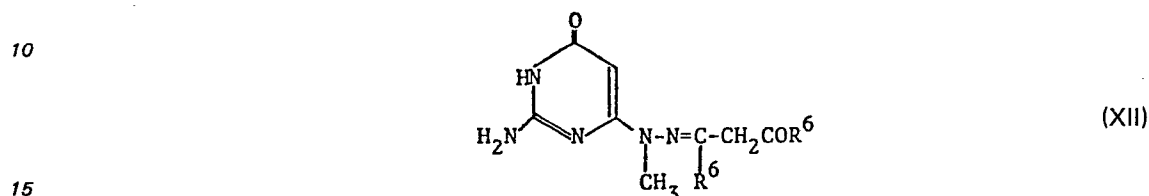


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where R^6 is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group, then the dicarbonyl compound will be a symmetrical 1,3-diketone of formula (XI):

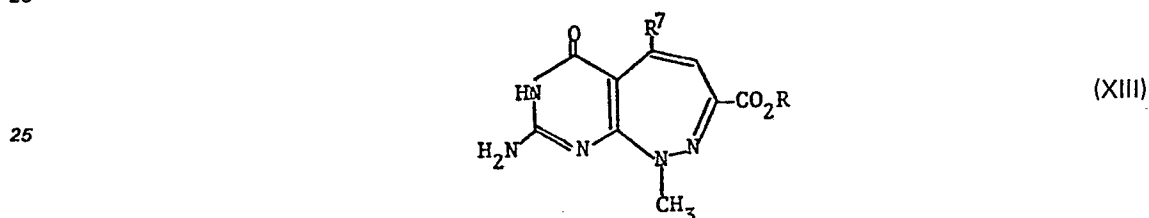


where R^6 is as defined above. In this case the intermediate will be a compound of formula (XII):

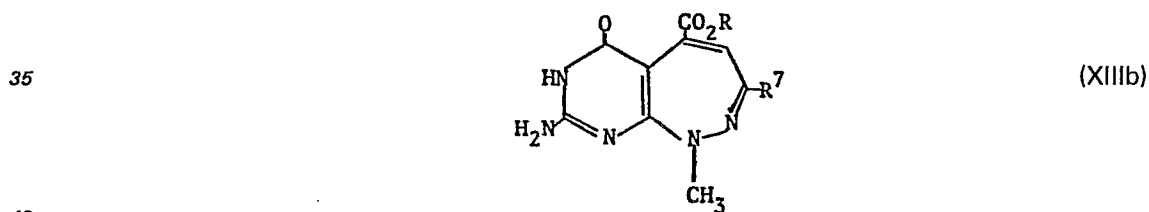


where R^6 is as defined above. Compounds of formula (XII) are novel.

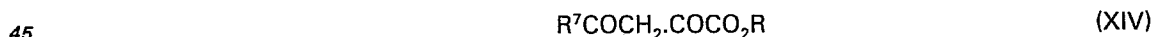
When, in the compounds of formula (I), R^1 is a lower alkyl group and R^2 is a group CO_2R in which R is a lower alkyl group, viz a compound of formula (XIIIa):



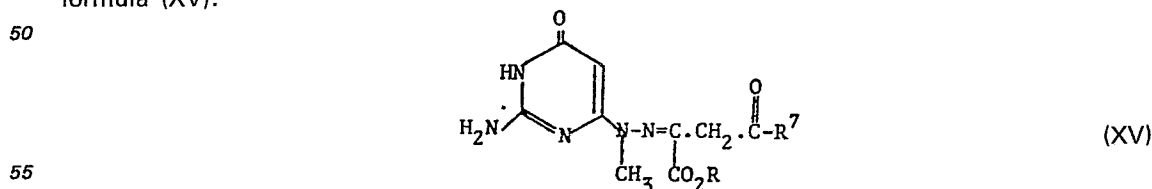
30 where R is as hereinbefore defined and R^7 is a lower alkyl group or, alternatively R^1 is a group CO_2R where R is a lower alkyl group and R^2 is a lower alkyl group, viz a compound of formula (XIIIb):



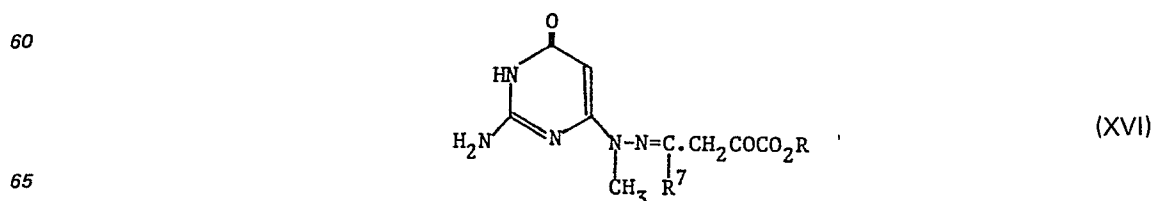
when R and R^7 are as defined above, the diketo compound will be an α,γ -diketo ester of formula (XIV):



where R and R^7 are as defined above. The reaction of a compound of formula (XIV) with the compound of formula (IV) always leads to a mixture of condensation products, believed to be compounds of formula (XV):



where R and R^7 are as hereinbefore defined and formula (XVI):

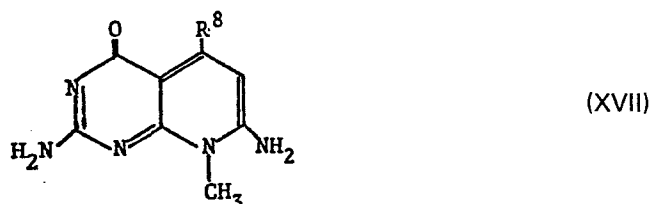


where R and R⁷ are as hereinbefore defined.

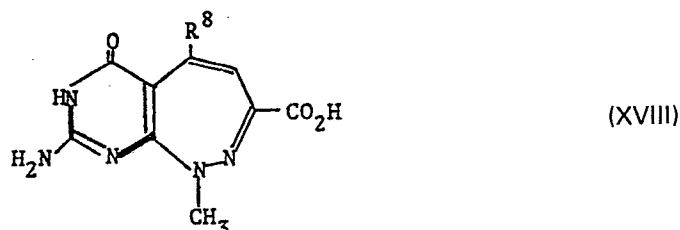
The compounds of formulae (XV) and (XVI) may either be separated from each other, for example by column chromatography before conversion into compounds of formulae (XIIIa) and (XIIIb) respectively, or preferably they may be cyclised together and the so produced isomeric compounds (XIIIa) and (XIIIb) may be separated from each other by methods known in the art for separating isomeric compounds, for example by column chromatography on, for instance, silica gel.

The compounds of formulae (XV) and (XVI) are novel compounds.

Compounds of formula (I) wherein R¹ is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group and R² is a group CO₂R in which R is as hereinbefore defined may be converted to pyrido[2,3-d]pyrimidines by saponification at room temperature followed by acidification to pH 2.0 to 5.0 to yield the carboxylic acid corresponding to the starting ester and subsequent heating to 100 to 150°C in an alkyl cellosolve (Cellosolve is a Trade Mark) or in dimethyl sulphoxide. Thus according to a further aspect of the present invention there is provided a method of making a compound of formula (XVIIa):



where R⁸ is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group which comprises heating in an alkyl cellosolve or in dimethyl sulphoxide, a compound of formula (XVIII):



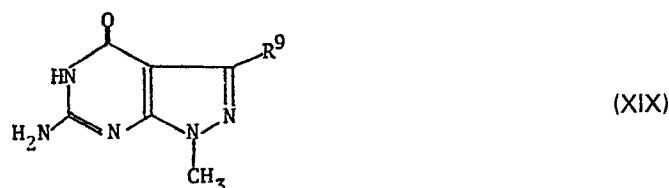
wherein R⁸ is as hereinbefore defined.

The compounds of formulae (XVII) and (XVIII) are novel.

The saponification of a compound of formula (I) wherein R¹ is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or, pyridyl group and R² is a group CO₂R in which R is as hereinbefore defined may conveniently be carried out using an aqueous alkali, for example aqueous sodium hydroxide. The subsequent acidification step which produces the corresponding compound of formula (XVIII) is conveniently performed using an aqueous acid, preferably an aqueous inorganic acid, for example hydrochloric acid.

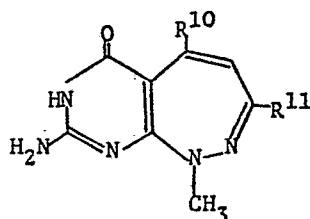
The alkyl cellosolve in which compounds of formula (XVIII) may be heated to produce compounds of formula (XVII) is preferably methyl cellosolve, and the heating should be carried out for 2—3 hours at 100 to 150°C, most preferably at 125°C. Preferably the compound of formula (XVIII) is separated from the reaction medium in which it is produced before it is heated to produce ring rearrangement.

Compounds of formula (I) wherein (a) R¹ is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group and R² is a group CO₂R in which R is a lower alkyl group; or (b) R¹ and R² are the same and each is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group may be converted to pyrazolo[3,4-d]pyrimidines by heating in aqueous acid at a temperature of greater than 70°C. Thus according to a further aspect of the present invention there is provided a process of making a compound of formula (XIX):



wherein R⁹ is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group which comprises heating in aqueous acid a compound of formula (XX):

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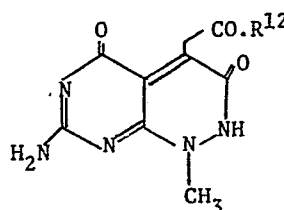


(XX)

wherein (a) R^{10} is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or a pyridyl group and R^{11} is a group CO_2R in which R is as hereinbefore defined; or (b) R^{10} and R^{11} are the same and each is a lower alkyl group, a phenyl group (optionally substituted with one or more hydroxy or lower alkoxy groups) or, pyridyl group.

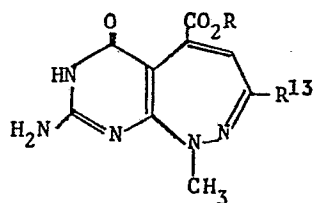
The acid for use in this reaction is conveniently an aqueous inorganic acid, for example hydrochloric acid, having a strength in the range of 0.5 to 2.0M. Normally the reaction reaches completion after 2 or 3 hours.

Compounds of formula (I) wherein R^2 is a lower alkyl group and R^1 is a group CO_2R in which R is as hereinbefore defined may be converted to pyrimido[4,5-c]pyridazines by treatment with aqueous acid at room temperature. Thus according to yet a further aspect of the present invention there is provided a method of making a compound of formula (XXI):



(XXI)

wherein R^{12} is a lower alkyl group which comprises treating with aqueous acid at room temperature a compound of formula (XXII):



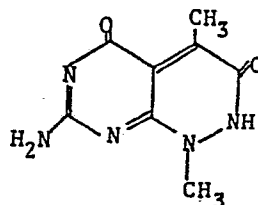
(XXII)

wherein R^{13} is a lower alkyl group and R is as hereinbefore defined.

It should be noted that R^{13} in formula (XXII) becomes R^{12} in formula (XXI).

The conditions for this hydrolysis reaction should be mild i.e. carried out without heating, and the acid, which is preferably an inorganic aqueous acid, for example hydrochloric acid, should be added until the reaction medium reaches a pH of 3 or less. Normally the reaction is completed within 2 or 3 days.

The compounds of formula (XXI) are novel. The compounds of formula (XXI) may themselves be further converted to a different pyrimido[4,5-c]pyridazine by boiling in a hydroxylic solvent, for example methyl cellosolve. Thus there is provided a method of making a compound of formula (XXIII):



(XXIII)

which comprises heating a compound of formula (XXI) in boiling methyl cellosolve. Normally the heating is carried out for up to 24 hours.

The compound of formula (XXIII) is novel.

It should be noted that regardless of the nature of 12 in the substituent at the 4-position in formula (XXI), cleavage normally results in the compound of formula (XXIII) which has a methyl group in its 4-position.

All the starting materials specified above for the various syntheses may be prepared by standard methods taught in the art.

As stated hereinabove the compounds of formula (II) or their tautomers, or salts thereof not only inhibit the growth of micro-organisms to some extent but also unexpectedly act with a potentiating effect when combined with a 2,4-diamino-5-benzylpyrimidine or with a sulphonamide, or with a combination of both these types of antimicrobial agents.

There may be provided a composition for treating microbial infections, comprising an effective potentiating amount of a compound of formula (II) in combination with an effective amount of a 2,4-diamino-5-benzylpyrimidine, or a sulphonamide, or both.

The microbial infections against which the combinations of this invention are effective are protozoal or bacterial infections caused by those microorganisms which synthesise at least a substantial part of their tetrahydrofolate co-factor requirements. More specifically these infecting microorganisms are those which adequately absorb the pharmaceutical combinations disclosed herein and further are those in which these combinations have a synergistic effect in interfering with the *de novo* synthesis of the required tetrahydrofolate co-factors.

Also as stated hereinabove the compounds of formula (III), their tautomers and their salts not only inhibit the growth of protozoa but also unexpectedly act with a potentiating effect when combined with a combination of a 2,4-diamino-5-benzylpyrimidine and a sulphonamide.

Thus may be provided a composition for treating protozoal infections comprising an effective potentiating amount of a compound of formula (III) in combination with an effective amount of a combination of a 2,4-diamino-5-benzylpyrimidine and a sulphonamide.

In accordance with the above, the term "an effective amount" used in conjunction with the terms a 2,4-diamino-5-benzylpyrimidine and a sulphonamide means either (a) an amount of the 2,4-diamino-5-benzylpyrimidine or sulphonamide which is effective to a degree as an antimicrobial agent in its own right but which is potentiated by the use of a compound of formula (II), or its tautomer, or salt thereof or (b) an amount of the 2,4-diamino-5-benzylpyrimidine or sulphonamide which is ineffective as an antimicrobial agent but which when combined with a compound of formula (II), or its tautomer, or salt thereof, provides a composition which is an effective antimicrobial agent. An "effective potentiating amount" means an amount of the compound of formula (II), or formula (III), or its tautomer or salt thereof which increases the activity of a 2,4-diamino-5-benzylpyrimidine and/or a sulphonamide so as to provide a greater antimicrobial effectiveness for the whole combination.

The compounds of formula (II) or (III) either for use alone or in combination with a 2,4-diamino-5-benzylpyrimidine and/or a sulphonamide may be presented in association with a carrier in pharmaceutical formulations suitable for parenteral, topical, rectal or oral administration. The formulations for oral or rectal administration are advantageously presented in discrete units, such as tablets, capsules, cachets, ampoules or suppositories each containing a predetermined amount of compound, but may also be presented as a powder, as granules, as a solution or suspension in an aqueous or non-aqueous liquid, or as an ointment or paste for topical administration. For parenteral use, the formulations incorporating an aqueous or non-aqueous liquid carrier must be sterile and be presented in sealed containers. The formulations may be made by any of the known methods and may include one or more of the following accessory ingredients: diluents, solutes to render the solution isotonic with the blood, buffers, flavouring, binding, dispersing, surface-active, thickening, lubricating and coating materials, preservatives, bacteriostats, antioxidants, suppository and ointment bases, and any other acceptable excipients.

Thus there may be provided a pharmaceutical formulation comprising a compound of formula (II) or (III) alone, or in combination with a 2,4-diamino-5-benzylpyrimidine and/or a sulphonamide, in admixture with a pharmaceutically acceptable carrier. Such pharmaceutical formulations may be prepared by admixing the compound of formula (II) or (III) alone, or in combination with a 2,4-diamino-5-benzylpyrimidine and/or a sulphonamide with a carrier by known techniques.

Any of the aforementioned antimicrobial compounds may be presented in the form of their pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts are those derived from mineral or organic acids, for example hydrochloric acid, hydrobromic acid, sulphuric acid, acetic acid, citric acid, tartaric acid, lactic acid, maleic acid, or salicylic acid, or especially for the sulphonamide, of a base, such as sodium or potassium hydroxide. Salts which are not pharmaceutically acceptable may be rendered so by a conventional metathetical reaction.

Humans and other animals suffering from microbial infections may be treated by administering a non-toxic effective antimicrobial treatment amount of a compound of formula (II) or (III), or preferably administering a pharmaceutical formulation comprising said amount of a compound of formula (II) or (III) and a pharmaceutically acceptable carrier, to the infected human or other animal.

Humans and other animals suffering from microbial infections may be treated by administering a composition comprising an effective potentiating amount of a compound of formula (II) or (III), in combination with an effective amount of a 2,4-diamino-5-benzylpyrimidine or a sulphonamide, or both, or preferably administering a pharmaceutical formulation comprising the said composition and a pharmaceutically acceptable carrier, to the infected human or other animal.

A suitable dose range for a compound of formula (II) or formula (III) (either alone or with a

sulphonamide and/or pyrimidine derivative) for the treatment of microbial infections lies in the range of 1 mg to 100 mg/kg body weight.

For compounds of formula (III) when used incorporated in animal feeds for the treatment or prophylaxis of protozoal infections a suitable dose range is from 100 to 400 ppm of active ingredient.

Further advantages of the present invention can be ascertained from the following Examples which should not be construed as limiting the scope of the invention in any way.

Example 1

8-Amino-3,5-diphenyl-1-methyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one



To a stirred, refluxing mixture of 6-(1-methylhydrazino)isocytosine hemihydrate (1.00 g) in methanol (100 ml) was added 1,3-diphenyl-1,3-propanedione (2.05 g). After 236 hours the orange solution was allowed to cool to room temperature.

The solution was concentrated under vacuum onto silica gel (5.0 g) that was added to a column of silica gel (90 g) in hexane. Elution of the column with benzene, a 3:1 benzene:chloroform mixture, chloroform, and ethyl acetate effected separation of the desired product that came off in the 3:1 benzene:chloroform mixture, chloroform, and ethyl acetate, yield 1.01 g. Yellow solid was obtained after recrystallisation from carbon tetrachloride, yield 0.64 g (29%); mp 205—220° dec.; nmr (DMSO- d_6) δ 3.12 (s, 3H), 6.69 (s, 1H), 6.77 (br. s, 2H), 7.2—7.6 (m, 8H), 7.6—8.0 (m, 2H), 10.6 (br. s, 1H); uv λ_{max} (CH₃OH) 255.5 nm (ϵ 22,200), 285 (26,400), 385 (700), Mass spectrum (210°C); M, m/e 343. The following accurate mass was determined: 343.1437 (C₂₀H₁₇N₅O).

Anal. Calcd. for C₂₀H₁₇N₅O·0.022 CCl₄; C, 69.35%; H, 4.94%; N, 20.20%; Cl, 0.90%. Found: C, 69.43%; H, 5.09%; N, 20.15%; Cl, 0.90%.

Example 2

8-Amino-1,3,5-trimethyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one



To a stirred, refluxing mixture of 6-(1-methylhydrazino)isocytosine hemihydrate (1.00 g) in methanol (100 ml) was added acetylacetone (0.913 g). After 48 hours the resulting orange solution was allowed to cool to room temperature and stand overnight.

The solution was concentrated by boiling to 25 ml and was then allowed to cool to room temperature. After 2 hours the slightly cloudy solution was filtered, and the clear filtrate was concentrated by boiling to 15 ml after which time it was allowed to cool to room temperature and stand overnight. No crystallisation occurred until after 24 hours when the solution was suddenly jarred. After an additional 2 hours yellow crystals were collected, washed quickly with methanol (5 ml) and hexane (10 ml), and dried under vacuum at 75°C to yield the desired product 0.541 g (41%); mp 250—253° dec; nmr (DMSO- d_6) δ 1.84 (s, 3H), 2.06 (d, 3H, J=1Hz), 2.88 (s, 3H), 5.89 (q, 1H, J=1Hz), 6.67 (br. s, 2H), 10.58 (br. s, 1H). On a different batch of product a Nuclear Overhauser Effect of 25% was observed for the aromatic



quartet at 5.89 δ upon irradiation of the aromatic —CH₃ doublet at 2.06 δ . A Nuclear Overhauser Effect of 19% was observed for the aromatic



quartet at 5.89 δ upon irradiation of the ring —CH₃ singlet at 1.84 δ . A coupling experiment showed that absorption at 2.065 and 5.895 were coupled to each other; uv λ_{max} (CH₃OH) 233.5 nm sh (ϵ 11,400), 267 (17,900), 302 (9,900), 348 (1,300); pKa 9.11 and 3.27. Mass spectrum of a different batch: M, m/e 219, 43%; m/e 178, 100%.

Anal. Calcd. for C₁₀H₁₃N₅O: C, 54.78%; H, 5.98%; N, 31.95%. Found: C, 54.96%; H, 5.99%; N, 31.72%.

Example 3

8-Amino-3-carbomethoxy-5-(3-hydroxyphenyl)-1-methyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one



5

To a stirring, refluxing mixture of 6-(1-methylhydrazino)isocytosine hemihydrate (1.76 g) in methanol (176 ml) was added methyl m-hydroxybenzoylpyruvate (2.88 g). After 22 hours precipitated solid was collected from the hot mixture, yield 0.282 g.

The filtrate was brought to boiling and was concentrated to 75 ml when precipitated solid was noticed to be present. The mixture was allowed to cool to room temperature and stand for several hours. Brownish-orange solid was collected, washed with two portions of methanol (10 ml each), and dried under vacuum at 80°C to yield the desired product 1.80 g (49%): mp > 275° dec; nmr (DMSO- d_6) δ 3.11 (s, 3H), 3.79 (s, 3H), 6.50 (s, 1H), 6.6—7.4 (m, 6H), 9.22 (br. s, 1H), 10.77 (br. s, 1H); uv λ max (CH₃OH) 244.5 nm (ϵ 19,600), 280.5 sh (16,700), 305 (20,500), 424 (700).

15 *Anal.* Calcd. for C₁₆H₁₅N₅O₄: C, 56.30%; H, 4.43%; N, 20.52%. Found: C, 56.34%; H, 4.48%; N, 20.56%.

Example 4

8-Amino-3-carbomethoxy-1-methyl-5-(3-pyridyl)-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one

20



To a stirred, refluxing mixture of 6-(1-methylhydrazino)isocytosine hemihydrate (1.00 g) in methanol (100 ml) was added methyl 2,4-dioxo-4-(3-pyridyl)butyrate (1.51 g). After 17½ hours brown solid was collected from the hot mixture, washed with two portions of methanol (10 ml each), and dried under vacuum at 75°C to yield the desired product 1.11 g (56%): mp 258—272° dec; nmr (DMSO- d_6) δ 3.11 (s, 3H), 3.79 (s, 3H), 6.56 (s, 1H), 6.98 (br. s, 2H), 7.1—7.8 (m, 2H), 8.3—8.6 (m, 2H), 10.9 (br. s, 1H); nmr (CF₃COOH) δ 3.53 (s, 3H), 4.12 (s, 3H), 7.13 (s, 1H), 8.0—8.3 (m, 1H), 8.5—9.0 (m, 3H); uv λ max (CH₃OH) 244.5 nm (ϵ 21,800), sh (16,500), 307.5 (17,500), 454 (900).

30 *Anal.* Calcd. for C₁₅H₁₄N₆O₃: C, 55.21%; H, 4.32%; N, 25.76%. Found: C, 55.19%; H, 4.29%; N, 25.84%.

Example 5

8-Amino-3-carbomethoxy-1-methyl-5-phenyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one

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To a stirred mixture of 6-(1-methylhydrazino)isocytosine hemihydrate (1.00 g) in absolute ethanol at 65—70° was added ethyl benzoylpyruvate (2.01 g). After 101 hours some precipitated solid was collected from the hot mixture. After a further ½ hour an additional small amount of solid was filtered from cooled filtrate.

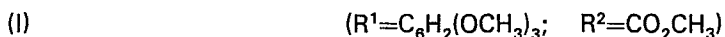
After the filtrate had stood overnight, precipitated pale orange solid was collected, washed with 5 ml of chloroform and 10 ml of hexane, and dried under vacuum at 75° to yield the desired product 0.796 g. Yellow solid was obtained after two recrystallisations from benzene, yield 0.200 g (9%): mp 240.5—242.5° dec; nmr (DMO- d_6) δ 1.28 (t, 3H), 3.12 (s, 3H), 4.26 (q, 2H), 6.52 (s, 1H), 6.89 (br. s, 2H), 7.28 (br. s, 5H), 10.7 (br. s, 1H); uv λ max (CH₃OH) 243.5 nm (ϵ 19,400), 294 (20,200), 419.5 (600). Mass spectrum (180°C): M, m/e 339, 35%; m/e 240, 100% (M—C₂H₅O₂CCN). The following accurate mass was determined: 240.1008 (C₁₃H₁₂N₄O). The most prominent metastable ion for the molecular ion was m/e 339 → m/e 240.

50 *Anal.* Calcd. for C₁₇H₁₇N₅O₃ · 0.20 C₆H₆: C, 61.58%; H, 5.17%; N, 19.73%. Found: C, 61.72%; H, 5.29%; N, 19.88%.

Example 6

8-Amino-3-carbomethoxy-1-methyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one

55



To a stirred, refluxing mixture of 6-(1-methylhydrazino)isocytosine hemihydrate (1.00 g) in methanol (100 ml) was added methyl 3,4,5-trimethoxybenzoylpyruvate (2.70 g). After 40 hours precipitated solid was collected from the hot mixture to yield 0.360 g of product.

The filtrate was concentrated under vacuum to 2.8 g of solid that was stripped onto silica gel (5.0 g) that was added to a column of silica gel (90 g) in hexane. Elution of the column with benzene, benzene:chloroform mixtures, chloroform, a 1:1 chloroform-ethyl acetate mixture, ethyl acetate, and finally a 9:1 ethyl acetate:methanol mixture effected separation of the desired product that came off in

the 9:1 ethyl acetate:methanol mixture yield 1.57 g. Tiny, orange crystals were obtained after recrystallisation from methanol to yield 1.15 g (45%) of product; mp 258—260° dec.; nmr (DMSO- d_6) δ 3.09 (s, 3H), 3.67, 3.74, and 3.77 (overlapping s's, 12H), 6.47 (s, 2H), 6.51 (s, 1H), 6.87 (br. s, 2H), 10.8 (br. s, 1H); uv λ max (CH₃OH) 239 nm sh (ϵ 19,900), 307.5 (24,600), 421 (800).

5 Mass spectrum of a different batch (210°C): M, m/e 415, 56%; m/e 330, 57%; m/e 315, 100%.
Anal. Calcd. for C₁₉H₂₁N₅O₆: C, 54.93%; H, 5.10%; N, 16.86%; Found: C, 54.99%; H, 5.15%; N, 16.82%.

The following compounds were prepared in a manner similar to that of Example 6:—

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Example 7

8-Amino-3-carbethoxy-1-methyl-5-(2,4-dimethoxyphenyl)-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one

15

(I) (R¹=C₆H₃(OCH₃)₂; R²=CO₂C₂H₅)

Refluxing absolute ethanol was used as solvent. Precipitated solid was collected after 42 hours. Product was eluted from a column with 9:1 ethyl acetate: methanol.

20 Yield after recrystallisation from benzene (28%): mp 187—196° dec.; nmr (DMSO- d_6) δ 1.27 (t, 3H), 3.09 (s, 3H), 3.64 (s, 3H), 3.77 (s, 3H), 4.24 (q, 2H), 6.4—7.1 (m, 6H), 10.5 (br s, 1H); uv λ max (CH₃OH) 246.5 nm (ϵ 20,500), 311.5 (21,700), 345.5 sh (5,100) 420 sh (900).

Anal. Calcd. for C₁₉H₂₁N₅O₅ · 0.43 C₆H₆: C, 59.86%; H, 5.49%; N, 16.18%. Found: C, 59.89%; H, 5.54%, N, 16.33%.

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Example 8

8-Amino-3-carbethoxy-1-methyl-5-(3,4-dimethoxyphenyl)-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one

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(I) R¹=C₆H₃(OCH₃)₂; R²=CO₂C₂H₅)

Absolute ethanol (65—70°C) was used as solvent. Precipitated solid was collected after 112 hours. Product was eluted from a column with ethyl acetate. Yield after dissolution in ethyl acetate and precipitation with hexane (7%): mp 162° dec; nmr (DMSO- d_6) δ 1.27 (t, 3H), 3.10 (s, 3H), 3.72 (s, 3H), 3.75 (s, 3H), 4.24 (q, 2H), 6.50 (s, 1H), 6.6—7.2 (m, 5H), 10.8 (br, s, 1H); uv λ max (CH₃OH) 245 nm (ϵ 20,800), 309 (21,900).

35 *Anal.* Calcd. for C₁₉H₂₁N₅O₅ · 0.1 CH₃CO₂C₂H₅ · 0.4 H₂O: C, 56.09%; H, 5.48%; N, 16.86%. Found: C, 56.16%; H, 5.23%; N, 16.86%.

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Example 9

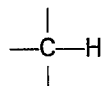
8-Amino-3-carbethoxy-1,5-dimethyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one

(I) (R¹=CH₃; R²=CO₂C₂H₅)

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To a stirred, refluxing mixture of 6-(1-methylhydrazino)isocytosine hemihydrate (5.00 g) in methanol (500 ml) was added ethyl acetylpyruvate (5.78 g). After 65 hours the solution was concentrated by boiling to 40 ml and was allowed to stand overnight.

50 The solution was concentrated under vacuum to 9.7 g of pasty solid that was stripped onto silica gel (10 g) that was then added to a column of silica gel (400 g) in hexane. Elution of the column with a 1:1 benzene:chloroform mixture, chloroform, chloroform:ethyl acetate mixtures, ethyl acetate, and a 4:1 ethyl acetate:methanol mixture effected separation of desired product that came off in ethyl acetate, yield 3.95 g. Orange crystals were obtained after recrystallisation from ethyl acetate, yield 2.52 g (30%): mp 244—245° dec; nmr (DMSO- d_6) δ 1.24 (t, 3H, J=7.5Hz), 2.12 (d, 3H, J=1Hz), 3.03 (s, 3H), 4.22 (q, 2H, J=7.5Hz), 6.18 (q, 1H, J=1Hz), 6.83 (br, s, 2H), 10.8 (br s, 1H). On a different
 55 batch of product, a Nuclear Overhauser Effect of 32% was observed for the aromatic



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quartet at 6.18 δ upon irradiation of the aromatic —CH₃ doublet at 2.12 δ ; uv of a different batch λ max (CH₃OH) 245 nm (ϵ 17,200), 284 sh (11,300), 306(14,100), 410(600). Mass spectrum of a different batch (155°C): M, m/e 277, 29%; m/e 178, 100% (M—NCCO₂C₂H₅). The following accurate masses were determined 277.1163 (C₁₂H₁₅N₅O₃), 178.0843 (C₈H₁₀N₄O).

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0 000 541

Anal. Calcd. for $C_{12}H_{15}N_5O_3$: C, 51.98%; H, 5.45%; N, 25.26%. Found: C, 51.91%; H, 5.45%; N, 25.18%.

Example 10

5 8-Amino-5-carbethoxy-1,3-dimethyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one

(I) $(R^1=CO_2C_2H_5; R^2=CH_3)$

The isomer of the compound just described was prepared and isolated in the same experiment. This product came off the column in ethyl acetate and a 4:1 ethyl acetate:methanol mixture after its isomer (previous Example) had come off, yield 3.13 g. Pale yellowish-orange crystals were obtained after two recrystallisations from carbon tetrachloride, yield 0.942 g (11%); mp 196° dec; nmr (DMSO- d_6) δ 1.16 (t, 3H), 1.93 (s, 3H), 2.91 (s, 3H), 4.06 (q, 2H), 6.39 (s, 1H), 6.83 (br, s, 2H), 10.8 (br, s, 1H), uv of a different batch λ max (CH₃OH) 241.5 nm (ϵ 14,500), 295 (14,200), 305.5 sh (13,100), 387 (900). Mass spectrum of a different batch (160°): M, m/e 277, 70%; m/e 236, 100% (M-CH₃CN). The following accurate masses were determined: 277.1170 ($C_{12}H_{15}N_5O_3$), 236.0902 ($C_{10}N_{12}N_4O_3$).

Anal. Calcd. for $C_{12}H_{15}N_5O_3 \cdot 0.45 CCl_4$: C, 43.15%; H, 4.36%; N, 20.21%; Cl, 18.24%. Found: C, 42.87%; H, 4.38%; N, 20.43%; Cl, 18.07%.

Example 11

20 2,7-Diamino-8-methyl-5-phenylpyrido [2,3-d]pyrimidin-4(8H)-one

(XVII) $(R^8=C_6H_5)$

To 8-amino-3-carbethoxy-1-methyl-5-phenyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one (2.9 g) was added N NaOH (100 ml), and a solution resulted after the mixture was swirled. After 45 minutes the solution was acidified with conc. HCl until pH 2.5—3.0 was reached. Precipitated yellow solid was collected, washed with water (3×15 ml), and dried under vacuum at $70^\circ C$, yield 2.4 g of product. This solid was shown by nmr to be the corresponding carboxylic acid of the starting ester.

A 2.3 g sample of solid was suspended in boiling methyl cellosolve (175 ml), and the resulting dark brownish-red solution quickly became orangish-yellow in colour. The solution was concentrated to 130 ml when solid precipitated. The mixture was allowed to cool to room temperature and stand for 2 hours. Off-white solid was collected, washed with methyl cellosolve (2×15 ml) and methanol (4×15 ml), and dried under vacuum at $70^\circ C$, yield 1.28 g (49%) of product: mp $> 300^\circ$; nmr (CF₃COOH) δ 4.08 (s, 3H), 6.68 (s, 1H), 7.0—7.8 (m, 7H); nmr (DMSO- d_6) δ 3.64 (s, 3H), 5.93 (s, 1H), 6.76 (br, s, 2H), 7.1—7.4 (m, 5H), 7.8* (very br s, 2H); uv λ max (CH₃OH) 216 nm (ϵ 35,300), 258 sh (9,400), 290.5 (13,000), 348 (17,400). Mass spectrum (250°): M, m/e 267, 100%; m/e 266, 26%; m/e 140, 10%. The following accurate mass was determined: 267.1120 ($C_{14}H_{13}N_5O$).

Anal. Calcd. for $C_{14}H_{13}N_5O \cdot 0.08 CH_3OCH_2CH_2OH \cdot 0.2 H_2O$: C, 61.75%; H, 5.11%; N, 25.29%. Found: C, 61.76%; H, 5.30%; N, 25.32%.

* This chemical shift was estimated by guessing the midpoint of a very broad integral.

Example 12

45 2,7-Diamino-8-methyl-5-(3-hydroxyphenyl)pyrido [2,3-d]-pyrimidin-4(8H)-one

(XVIII) $(R^8=C_6H_4OH)$

To 8-amino-3-carbomethoxy-1-methyl-5-(3-hydroxyphenyl)-1H-pyrimido [4,5-c]-1,2-diazepin-6(7H)-one 4.79 g) was added N NaOH (190 ml), and a solution resulted after the mixture was swirled. After 1 hour 15 minutes the solution was acidified with conc. HCl until pH 1 was reached. Precipitated solid was collected, washed with water (3×10 ml), and dried under vacuum at $80^\circ C$ to yield 4.49 g of product. This solid was shown by nmr to be the corresponding carboxylic acid of the starting ester.

The carboxylic acid was boiled for $2\frac{1}{2}$ hours in methyl cellosolve (50 ml), and product was collected from a hot mixture, washed with methyl cellosolve (15 ml) and methanol (3×10 ml), and dried under vacuum at 80° , yield (60): mp $> 300^\circ$; nmr (CF₃COOH): δ 4.07 (s, 3H), 6.69 (s, 1H), 7.0—7.6 (m, 6H); uv λ max (CH₃OH) 217.5 nm (ϵ 42,300), 247 sh (10,700), 292 (14,300), 338 weak sh (16,200), 347 (18,400).

Anal. Calcd. for $C_{14}H_{13}N_5O_2$: C, 59.35%; H, 4.63%; N, 24.72%. Found: C, 59.24%; H, 4.65%; N, 24.70%.

Example 13

65 2,7-Diamino-8-methyl-4-(3,4,5-trimethoxyphenyl)pyrido[4,5-d]pyrimidin-4(8H)-one

(XIX) $(R^8=C_6H_2(OCH_3)_3)$

Following the general procedure of Example 11, the above compound was synthesised.

Yield (55%): mp > 300°; nmr (CF₃COOH) δ 4.00 (s, 6H), 4.09 (s, 6H), 6.73 (s, 1H), 6.76 (s, 2H), 7.26 (br, s, 2H); uv λ max (CH₃OH) 261.5 nm (ϵ 10,400), 293 (14,000), 339 weak sh (16,900), 348 (18,800). Mass spectrum (260°): M, m/e 357, 100%; m/e 342, 16% (M—CH₃); m/e 327, 4% (M—CH₂O and M—C₂H₆); m/e 317, 1% (M—C₂H₂N); m/e 316, 1% (M—C₂H₃N).

The following accurate masses were determined: 357.1432 (C₁₇H₁₉N₅O₄), 342.1203 (C₁₆H₁₆N₅O₄), 327.1333 (C₁₆H₁₇N₅O₃), 327.0969 (C₁₅H₁₃N₅O₄), 317.1265 (C₁₅H₁₇N₄O₄), 316.1164 (C₁₅H₁₆N₄O₄).

Anal. Calcd. for C₁₇H₁₉N₅O₄: C, 57.13%; H, 5.36%; N 19.60%. Found: C, 57.15%; H, 5.41%; N, 19.52%.

The reaction was shown to proceed through the intermediate carboxylic acid. This acid was identified by nmr and gave the following microanalysis: Calcd. for C₁₈H₁₉N₅O₆: C, 53.86; H, 4.77; N, 17.45%. Found: C, 53.90; H, 4.80; N, 17.34%.

Example 14

2,7-Diamino-5,8-dimethylpyrido[4,5-d]pyrimidin-4(8H)-one

(XVII)

(R⁸=CH₃)

Following the general procedure of Examples 11 and 12, the above compound was synthesised.

Yield (68%): mp > 300°; nmr (CF₃COOH) δ 2.81 (s, 3H), 4.00 (s, 3H), 6.65 (s, 1H), 6.96 (br, s, 2H); nmr (DMSO-d₆) δ 2.54 (s, 3H), 3.61 (s, 3H), 5.98 (s, 1H), 6.49 (br s, 2H), 7.5* (very br s, 2H); uv λ max (CH₃OH) 218 nm (ϵ 31,100), 253 (4,300), 290.5 (8,400), 327.5 sh (17,300), 337(20,400). Mass spectrum (250°C): M, m/e 205, 100%; m/e 165, 4% (M—C₂H₂N); m/e 164, 3% (M—C₂H₃N). The following accurate masses were determined: 205.0962 (C₉H₁₁N₅O), 165.0785 (C₇H₉N₄O), 164.0709 (C₇H₈N₄O).

Anal. Calcd. for C₉H₁₁N₅O: C, 52.67; H, 5.40; N, 34.13%. Found: C, 52.50; H, 5.40; N, 34.05%.

* This chemical shift was estimated by guessing the midpoint of a very broad integral.

Example 15

2,7-Diamino-8-methyl-5-(3-pyridyl)pyrido[4,5-d]-pyrimidin-4(8H)-one

(XVII)

(R⁸=3-pyridyl)

The above compound was synthesised following the general procedure of Examples 11 and 12.

Yield (27%): mp 301—304° dec; nmr (CF₃COOH) δ 4.12 (s, 3H), 6.85 (s, 1H), 7.69 (br s, 2H), 8.1—8.4 (m, 1H), 8.6—9.1 (m, 3H); uv λ max (CH₃OH) 215 nm (ϵ 34,800), 264.5 (11,300), 290 (10,800), 352.5 (15,900). Mass spectrum: M, m/e 268, 100%; m/e 240, 10%; m/e 228, 3% (M—C₂H₂N); m/e 227, 3% (M—C₂H₃N and M—CHN₂). The following accurate masses were determined: 268.1072 (C₁₃H₁₂N₆O), 228.0885 (C₁₁H₁₀N₅O), 2287.0923 (C₁₂H₁₁N₄O), 227.0811 (C₁₁H₉N₅O).

Anal. Calcd. for C₁₃H₁₂N₆O·0.24 CH₃OCH₂CH₂OH. 0.18 H₂O: C, 56.86%; H, 4.97%; N, 29.00%. Found: C, 56.82%; H, 5.08%; N, 29.05%.

Example 16

6-Amino-1,3-dimethyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one

(XIX)

(R⁹=CH₃)

A mixture of 8-amino-1,3,5-trimethyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one (219 mg) and N hydrochloric acid (3 ml) was heated using a hot water bath (> 85°C) for 2 hours. During that period a solution formed before crystals gradually began to separate. The mixture stood at room temperature overnight before the straw-coloured crystals were collected. The mixture with water and dried under vacuum (70°C) to yield 140 mg (78%) of analytically pure product: mp > 300°; nmr (DMSO-d₆) δ 2.28 (s, 3H), 3.60 (s, 3H), 6.52 (br s, 2H), 10.30 (br s, 1H); uv λ max (CH₃OH) 217 nm (ϵ 26,800), 254 (13,000). Mass spectrum (120°C, 70 ev): M, m/e 179, 100%.

Anal. Calcd. for C₇H₉N₅O: C, 46.92%; H, 5.06%; N, 39.09%. Found: C, 46.68%; H, 5.11%; N, 38.94%.

The following compounds (Examples 17, 18 and 19) were prepared by similar acid-catalysed hydrolysis conditions:

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Example 17

6-Amino-1-methyl-3-phenyl-1H-pyrazolo[4,5-d]pyrimidin-4(5H)-one

(XIX)

(R⁹=C₆H₅)

5

Method A

Starting material, 8-amino-3-carbomethoxy-1-methyl-5-phenyl-1H-pyrazolo[4,5-c]-1,2-diazepin-6(7H)-one; reaction time, 3 hours; yield after recrystallisation from ethanol, 43%; mp > 300°; nmr (DMSO-d₆) δ 3.73 (s, 3H), 6.65 (br s, 2H), 7.30—7.50 (m, 3H), 8.25—8.42 (m, 2H), 10.90 (br s, 1H);
10 uv λ max (CH₃OH) 246 nm (ε 29,900), 280 sh (8,400). Mass spectrum (170°, 70° ev): M, m/e 241, 100%.

Anal. Calcd. for C₁₂H₁₁N₅O: C, 59.74%; H, 4.60%; N, 29.03%. Found: C, 59.60%; H, 4.66%; N, 28.92%.

15 Method B

Starting material, 8-amino-3,5-diphenyl-1-methyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one; reaction time, 3 hours; yield after washing with ether, * 29%; spectral data same as that recorded above.

Anal. Found: C, 59.47%; H, 4.59%; N, 28.77%.

20 * A competing reaction produced 1,3-diphenyl-1,3-propanedione which was isolated from the ether wash in 50% yield.

Example 18

6-Amino-1-methyl-3-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,5-d]pyrimidin-4(5H)-one

25

(XIX)

(R⁹=C₆H₂(OCH₃)₃)

Starting material, 8-amino-3-carbomethoxy-1-methyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrimido-
30 {4,5-c}-1,2-diazepin-6(7H)-one; reaction time, 3 hours; yield after subsequent neutralization of the collected, crude product with 0.1N sodium hydroxide and recrystallisation from methanol, 40%; mp 183—184°; nmr (DMSO-d₆) δ 3.70 (s, 3H), 3.77 (s, 3H), 3.85 (s, 6H), 6.70 (br s, 2H), 7.87 (s, 2H), 10.43 (br s, 1H) plus methanol δ 3.19 (d, 3H, J=5 Hz), 4.04 (q, 1H, J=5Hz); uv λ max (CH₃OH) 222.5 nm (ε 33,200), 254.5 (24,100), 282 (14,800). Mass spectrum (160°, 70° ev): M, m/e 331, 100%*.

35 *Anal.* Calcd. for C₁₅H₁₇N₅O₄·CH₃OH: C, 52.89%; H, 5.83%, N, 19.27%. Found: C, 52.73%; H, 5.63%; N, 19.45%.

* This mass spectrum was obtained for a different batch prepared by the same method.

Example 19

6-Amino-3-(3-hydroxyphenyl)-1-methyl-1H-pyrazolo[4,5-d]pyrimidin-4(5H)-one-0.55
40 methanolate-0.16 hydrate

(XIX)

(R⁹=C₆H₄OH)

Starting material, 8-amino-3-carbomethoxy-5-(3-hydroxyphenyl)-1-methyl-1H-pyrimido[4,5-c]-
45 1,2-diazepin-6(7H)-one; reaction time, 2 hours. A solid impurity was removed by filtration before the crude product was subsequently precipitated from the mother liquor by neutralization with 4N sodium hydroxide solution, collected by filtration, washed with water, dried under vacuum (70°) and recrystallised twice from methanol; yield, * 21%; mp > 300°; nmr (DMSO-d₆) δ 3.74 (s, 3H), 6.67 m and 6.80 m (3H), 7.21 (t, 3H, J=8Hz), 7.81 m and 7.89 m (2H), 9.36 (s, 1H), 10.50 (br s, 1H) plus
50 methanol (0.5 mol) 3.19 (d, 3H, J=5Hz), 4.04 (q, 1H, J=5Hz) and water δ 3.30; uv λ max (CH₃OH) 248 nm (ε 26,400), 284 sh (8,800). Mass spectrum (150°, 70° ev): M, m/e 257, 100%.

Anal. Calcd. for C₁₂H₁₁N₅O₂·0.55 CH₃OH·0.16H₂O: C, 54.27%; H, 4.91%; N, 25.22%. Found: C, 54.25%; H, 4.82%; N, 25.20%.

* Other batches obtained during recrystallisation provided evidence for an overall yield of ca. 40%.

55

Example 20

4-Acetyl-7-amino-1-methylpyrimido[4,5-c]-pyridazine-3,5-(1H, 2H)-dione

(XXI)

(R¹²=CH₃)

60

A solution of 8-amino-5-carbomethoxy-1,3-dimethyl-1H-pyrimido[4,5-c]-1,2-diazepin-6(7H)-one (0.407 g) in a strongly acidic (pH ≤ 2), aqueous solution of HCl (1.3 ml) was allowed to stand for one day. Precipitated brownish-orange solid was collected, washed with water, and dried under vacuum at 75°C to yield 0.080 g of a product mixture.

65 After an additional three days brownish-orange solid in the filtrate was collected, washed with two

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portions of water (2 × 2.5 ml) and dried under vacuum at 75°C to yield 0.097 g (25%) of product: nmr (CF₃COOH) δ 2.60 (s, 3H), 4.28 (s, 3H), 4.85 (s, 2H), 6.92 (br s, 2H). Qualitative uv data and the nmr data suggested that the compound had the structure named above.

5

Example 21

7-Amino-1,4-dimethylpyrimido[4,5-c]pyridazine-3,5-(1H, 6H)-dione

(XXIII)

10

4-Actonyl-7-amino-1-methylpyrimido[4,5-c]pyridazine-3,5-(1H, 2H)-dione (0.054 g) was dissolved in boiling methyl cellosolve (1 l), and the solution was concentrated to 40 ml when precipitated solid was noticed to be present. The mixture was allowed to cool to room temperature and stand overnight. Yellowish-green solid was collected, washed with methyl cellosolve (10 ml) methanol (2 ml), and dried under vacuum at 75°C to yield 0.018 g (35%) of product: nmr (CF₃COOH) δ 3.03 (s, 3H), 4.26 (s, 3H), 6.83 (br s, 2H).

15

Example 22

The antibacterial activity against *Staphylococcus aureus* of trimethoprim (TMP) and sulphamethoxazole (SMX) alone and in combination with compounds of formula (II) were compared.

20

The results shown in the table are expressed as an activity index which is:—

ED₅₀ of SMX (or TMP) alone

ED₅₀ of SMX (or TMP) plus compound of formula (II)

25

the effect of the compounds being measured by viable counts after 24 hours incubation of 10⁶ organisms per ml.

In this test, the compounds of formula (II) were present at 50 µg/ml.

An activity index ≥ 2 is indicative of substantial potentiation:—

30

Compound of formula II		TMP	SMX
R ₃	R ₄		
C ₆ H ₅	CO ₂ C ₂ H ₅	2.0	>2.8
C ₆ H ₄ OH(3)	CO ₂ CH ₃	0.9	2.7

35

40

Example 23

The anti-coccidial activity of compounds of formula (III) alone and in combination with a mixture of diaveridine (DV) and sulphaquinoxaline (SQX) *in vitro* were tested according to the following procedure.

Using standard test procedures employing primary explant cultures of chick embryo liver cells and a suspension of *Eimeria Tenella* (Weybridge) sporozites the activity of the compounds was determined at various concentrations. In the above test, each test compound was used either alone or in combination with sulphaquinoxaline (0.01 µg/ml) and diaveridine (0.031 µg/ml). It should be noted that the minimum active anti-coccidial amounts of sulphaquinoxaline and diaveridine are 0.024 µg/ml and 0.098 µg/ml respectively.

45

The following results were obtained wherein activity is expressed as follows:—

50

5 = No parasite development

4 = 1—25% parasite development

3 = 26—50% parasite development

2 = 51—75% parasite development

1 = 76—95% parasite development

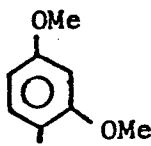
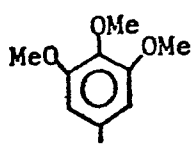
55

0 = 95—100% parasite development

60

65

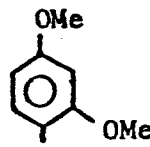
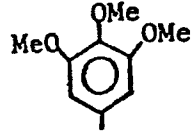
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Compound of formula (III)		Activity (concentration $\mu\text{g/ml}$)	
R ³	R ⁴	Alone	With DV/SOX
5 10 	CO ₂ C ₂ H ₅	4(100)	5(100)
		4(25)	5(25)
		2(6.25)	3(6.25)
		0(1.56)	2(1.56)
15 20 	CO ₂ CH ₃	3(100)	5(100)
		3(25)	4(25)
		3(6.25)	3(6.25)
		2(1.56)	3(1.56)
C ₆ H ₅	C ₆ H ₅	4(25)	4(25)

Example 24

Compounds of formula (III) were tested for anti-coccidial activity *in vivo* in combination with diaveridine (DV) and sulphaquinoxaline (SQX), each at 20 ppm, according to the following procedure.

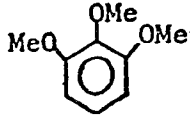
Groups of five, one week old chicks were each injected orally with 6,000 sporulated oocysts of the Weybridge strain of *Eimeria tenella*. Drugs were administered in LD 5 chick mash deficient in Vitamin K, beginning on the day of infection and continuing for 7 days. On the sixth day after infection, caecal lesions of surviving chicks were scored on a scale of 0, 1, 2 or 3 and any dead chicks were scored as 4. The following results were obtained:—

Compound of formula (III)		Concn. in diet (ppm)	Concn. DV in diet (ppm)	Concn. SQX in diet (ppm)	Lesion score
R ³	R ⁴				
—	—	—	20	20	2.2
45 	CO ₂ C ₂ H ₅	200	20	20	1.2
50 55 	CO ₂ CH ₃	200	20	20	0.6

Example 25

By standard techniques the LD₅₀ values of the following compounds of formula (I) were determined in mice.

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Compound of formula (I)		LD50 i.p. in mice
R ¹	R ²	
	CO ₂ CH ₃	>500 mg/kg
CH ₃	CH ₃	500 mg/kg

Example 26

(i) Tablets containing the following amounts of active ingredients are prepared by standard methods of pharmacy.

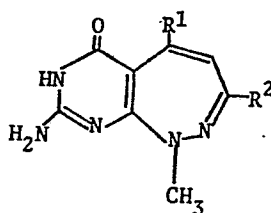
(A) Compound of Example 1	100 mg
(B) Compound of Example 3	50 mg
(C) Compound of Example 5	40 mg
Sulphamethoxazole	400 mg
Trimethoprim	80 mg

(ii) An animal feed containing the following active ingredients is prepared.

Compound of Example 6	200 ppm
Sulphamethoxazole	20 ppm
Trimethoprim	20 ppm

Claims

1. A compound of formula (I) or a tautomer or salt thereof



(I)

wherein

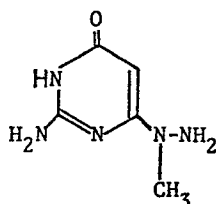
R¹ and R² are the same or different and are selected from a lower (C₁₋₄) alkyl group, a phenyl group (optionally substituted by one or more hydroxy or lower (C₁₋₄) alkoxy groups), a pyridyl group or a group —CO₂R in which R is a lower (C₁₋₄) alkyl group; provided that when R¹ is a group —CO₂R, R² is a lower (C₁₋₄) alkyl group.

2. A compound according to claim 1 wherein R¹ is a phenyl group optionally substituted by one or more hydroxy groups and R² is a group —CO₂R.

3. A compound according to claim 1 wherein R¹ is a phenyl group optionally substituted by one or more lower alkoxy groups and R² is selected from a phenyl group and a group —CO₂R.

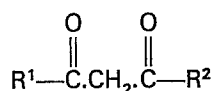
4. A method of preparing a compound of formula (I) as defined in claim 1 which comprises the reaction of a compound of formula (IV):

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(IV)

with a compound of formula (V):



(V)

wherein

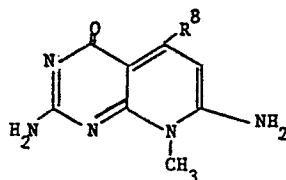
R¹ and R² are as defined in claim 1.

5. A pharmaceutical formulation comprising a compound of formula (I) as defined in either claim 2 or claim 3 together with an acceptable carrier therefor.

6. A method of making a pharmaceutical formulation, as defined in claim 5, which comprises admixing the compound of formula (I) with a carrier.

7. A pharmaceutical formulation according to claim 4 in unit dosage form.

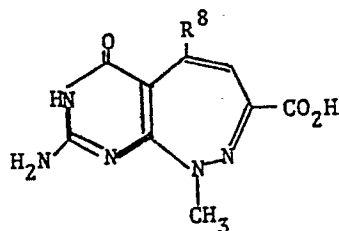
8. A method of preparing a compound of the formula (XVII) or a tautomer or salt thereof,



(XVII)

wherein

R⁸ is a lower (C₁-₄) alkyl group, a phenyl group (optionally substituted by one or more hydroxy or lower alkoxy groups) or a pyridyl group which method comprises heating in ethylene glycol monoalkyl ether or dimethylsulphoxide a compound of formula (XVIII):



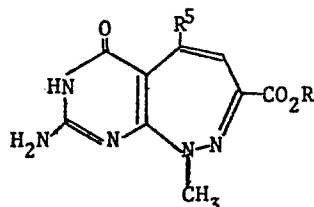
(XVIII)

wherein

R⁸ is as defined above.

9. A compound of formula (XVIII) as defined in claim 8.

10. A method of preparing a compound of formula (XVIII) as defined in claim 8 which comprises saponification of a compound of formula (VII):



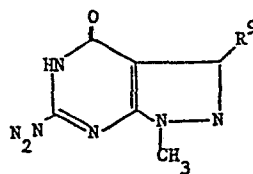
(VII)

wherein

R⁵ is selected from a lower (C₁-₄) alkyl group, a phenyl group (optionally substituted by one or more hydroxy or lower (C₁-₄) alkoxy groups) or a pyridyl group, and R is a lower (C₁-₄) alkyl group.

11. A method for the preparation of a compound of the formula (XIX) or a tautomer or salt thereof,

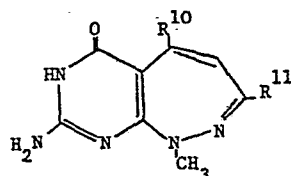
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(XIX)

wherein

R⁹ is selected from a lower (C₁₋₄) alkyl group, a phenyl group (optionally substituted by one or more hydroxy or lower (C₁₋₄) alkoxy groups) or a pyridyl group which method comprises heating in aqueous acid a compound of the formula (XX):

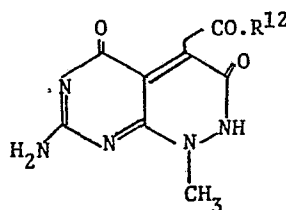


(XX)

wherein

R¹⁰ is selected from a lower (C₁₋₄) alkyl group, a phenyl (optionally substituted by one or more hydroxy or lower (C₁₋₄) alkoxy groups) or a pyridyl group and R¹¹ is selected from a lower (C₁₋₄) alkyl group, a phenyl group (optionally substituted by one or more hydroxy or lower alkoxy groups), a pyridyl group or a group —CO₂R where R is a lower (C₁₋₄) alkyl group provided that where R¹¹ is other than a group —CO₂R, R¹⁰ and R¹¹ are the same.

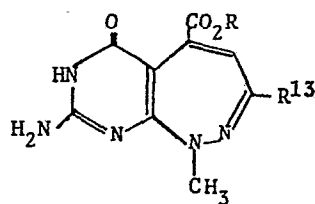
12. A method for the preparation of a compound of the formula (XXI), a tautomer or salt thereof,



(XXI)

wherein

R¹² is a lower (C₁₋₄) alkyl group which method comprises treating a compound of formula (XXII) with aqueous acid at room temperature,



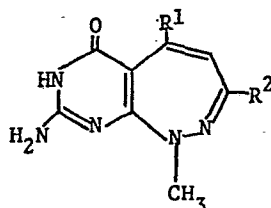
(XXII)

wherein

R and R¹³ are both lower (C₁₋₄) alkyl groups.

Patentansprüche

1. Verbindung der Formel (I) oder ein Tautomeres oder ein Salz davon



(I)

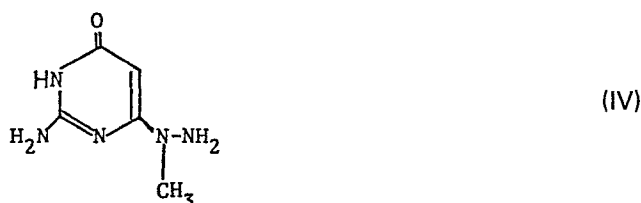
in der R¹ und R² gleichartig oder verschieden sind, und aus der Gruppe ausgewählt sind, die niedrig-

molekulare (C_{1-4})-Alkylgruppen, Phenylgruppen (die gegebenenfalls durch eine oder mehrere Hydroxygruppen oder niedrigmolekulare (C_{1-4})-Alkoxygruppen substituiert sind), Pyridylgruppen oder Gruppen der Formel $—CO_2R$, worin R für eine niedrigmolekulare (C_{1-4})-Alkylgruppe steht, umfaßt; mit der Maßgabe, daß wenn R^1 eine Gruppe der Formel $—CO_2R$ darstellt, R^2 eine niedrigmolekulare (C_{1-4})-Alkylgruppe bedeutet.

2. Verbindung nach Anspruch 1, dadurch gekennzeichnet, daß R^1 eine gegebenenfalls durch ein oder mehrere Hydroxygruppen substituierte Phenylgruppe und R^2 eine Gruppe der Formel $—CO_2R$ bedeuten.

3. Verbindung nach Anspruch 1, dadurch gekennzeichnet, daß R^1 eine gegebenenfalls durch eine oder mehrere niedrigmolekulare Alkoxygruppen substituierte Phenylgruppe und R^2 eine Phenylgruppe oder eine Gruppe der Formel $—CO_2R$ bedeuten.

4. Verfahren zur Herstellung einer Verbindung der Formel (I), wie sie in Anspruch 1 definiert ist, dadurch gekennzeichnet, daß man eine Verbindung der Formel (IV)



mit einer Verbindung der Formel (V)



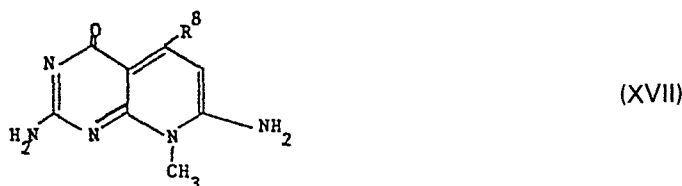
worin R^1 und R^2 die in Anspruch 1 angegebenen Bedeutungen besitzen umsetzt.

5. Pharmazeutische Zubereitung enthaltend eine Verbindung der Formel (I), wie sie in den Ansprüchen 2 oder 3 definiert ist und einen geeigneten Träger dafür.

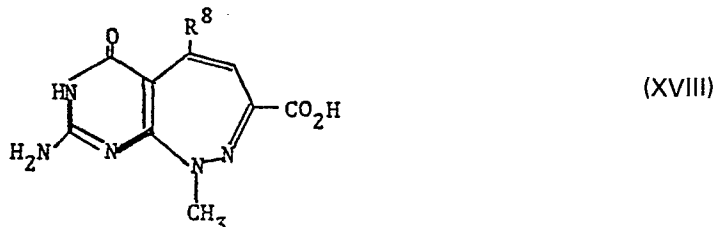
6. Verfahren zur Herstellung der pharmazeutischen Zubereitung nach Anspruch 5, dadurch gekennzeichnet, daß man die Verbindung der Formel (I) mit einem Träger vermischt.

7. Pharmazeutische Zubereitung nach Anspruch 1, dadurch gekennzeichnet, daß sie in Einheitsdosisform vorliegt.

8. Verfahren zur Herstellung einer Verbindung der Formel (XVII) oder eines Tautomeren oder eines Salzes davon



worin R^8 eine niedrigmolekulare (C_{1-4})-Alkylgruppe, eine Phenylgruppe (die gegebenenfalls durch eine oder mehrere Hydroxygruppen oder niedrigmolekulare Alkoxygruppen substituiert ist) oder eine Pyridylgruppe bedeutet, dadurch gekennzeichnet, daß man eine Verbindung der Formel (XVIII)

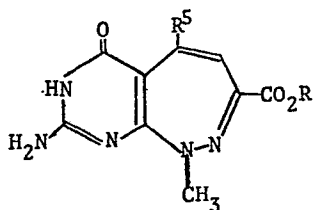


worin R^8 die oben angegebenen Bedeutungen besitzt, in Alkylcellosolve oder Dimethylsulfoxid erhitzt.

9. Verbindung der Formel (XVIII), wie sie in Anspruch 8 definiert ist.

10. Verfahren zur Herstellung einer Verbindung der Formel (XVIII), wie sie in Anspruch 8 definiert ist, dadurch gekennzeichnet, daß man eine Verbindung der Formel (VII)

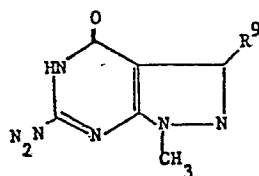
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(VII)

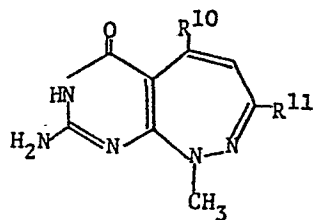
worin R^5 eine niedrigmolekulare (C_{1-4})-Alkylgruppe, eine Phenylgruppe (die gegebenenfalls durch eine oder mehrere Hydroxygruppen oder niedrigmolekulare (C_{1-4})-Alkoxygruppen substituiert ist) oder eine Pyridylgruppe und R eine niedrigmolekulare (C_{1-4})-Alkylgruppe bedeuten, verseift.

11. Verfahren zur Herstellung einer Verbindung der Formel (XIX) oder eines Tautomeren oder eines Salzes davon,



(XIX)

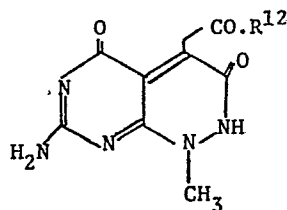
worin R^9 eine niedrigmolekulare (C_{1-4})-Alkylgruppe, eine Phenylgruppe (die gegebenenfalls durch eine oder mehrere Hydroxygruppen oder niedrigmolekulare (C_{1-4})-Alkoxygruppen substituiert ist) oder eine Pyridylgruppe bedeutet, dadurch gekennzeichnet, daß man eine Verbindung der Formel (XX)



(XX)

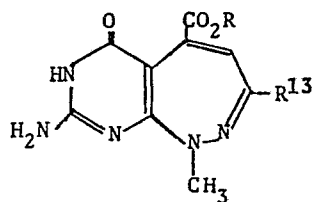
worin R^{10} eine niedrigmolekulare (C_{1-4})-Alkylgruppe, eine Phenylgruppe (die gegebenenfalls durch eine oder mehrere Hydroxygruppen oder niedrigmolekulare (C_{1-4})-Alkoxygruppen substituiert ist) oder eine Pyridylgruppe und R^{11} eine niedrigmolekulare (C_{1-4})-Alkylgruppe, eine Phenylgruppe (die gegebenenfalls durch eine oder mehrere Hydroxygruppen oder niedrigmolekulare Alkoxygruppen substituiert ist), eine Pyridylgruppe oder eine Gruppe der Formel $-\text{CO}_2\text{R}$, worin R für eine niedrigmolekulare (C_{1-4})-Alkylgruppe steht, mit der Maßnahme bedeuten, daß wenn R^{11} von der Gruppe der Formel $-\text{CO}_2\text{R}$ verschieden ist, R^{10} und R^{11} gleichartige Gruppen sind, in wässriger Säure erhitzt.

12. Verfahren zur Herstellung einer Verbindung der Formel (XXI), eines Tautomeren oder eines Salzes davon,



(XXI)

worin R^{12} eine niedrigmolekulare (C_{1-4})-Alkylgruppe bedeutet, dadurch gekennzeichnet, daß man eine Verbindung der Formel (XXII)

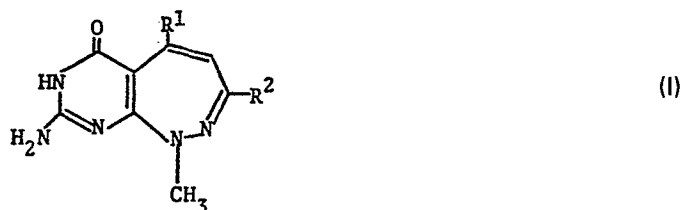


(XXII)

worin R und R^{13} jeweils niedrigmolekulare (C_{1-4})-Alkylgruppen bedeuten, bei Raumtemperatur mit einer wässrigen Säure behandelt.

Revendications

1. Composé de formule (I) ou tautomère ou sel correspondant:



dans laquelle R¹ et R² sont identiques ou différents et sont choisis entre un radical alcoyle inférieur (C₁₋₄), un radical phényle [éventuellement substitué par un ou plusieurs radicaux hydroxyle ou alcoxy inférieurs (C₁₋₄)], un radical pyridyle et un radical —CO₂R où R est un radical alcoyle inférieur (C₁₋₄), étant entendu que lorsque R¹ est un radical —CO₂R, R² est un radical alcoyle inférieur (C₁₋₄).

2. Composé suivant la revendication 1, dans lequel R¹ est un radical phényle éventuellement substitué par un ou plusieurs radicaux hydroxyle et R² est un radical —CO₂R.

3. Composé suivant la revendication 1, dans lequel R¹ est un radical phényle éventuellement substitué par un ou plusieurs radicaux alcoxy inférieurs et R² est choisi entre un radical phényle et un radical —CO₂R.

4. Procédé de préparation d'un composé de formule (I) tel que défini dans la revendication 1, qui comprend la réaction d'un composé de formule (IV):



avec un composé de formule (V):



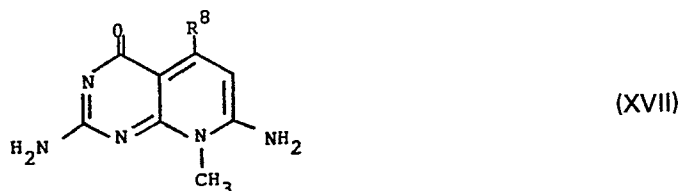
où R¹ et R² sont tels que définis dans la revendication 1.

5. Composition pharmaceutique comprenant un composé de formule (I) tel que défini dans la revendication 2 ou 3, outre un excipient acceptable.

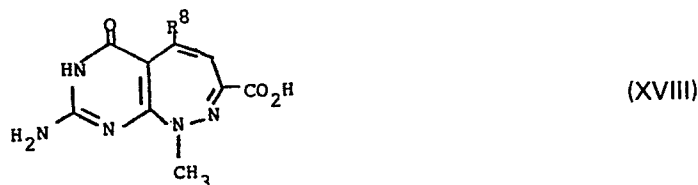
6. Procédé de préparation d'une composition pharmaceutique telle que définie dans la revendication 5, qui comprend le mélange du composé de formule (I) avec un excipient.

7. Composition pharmaceutique suivant la revendication 4 sous forme dosée unitaire.

8. Procédé de préparation d'un composé de formule (XVII) ou d'un sel ou tautomère correspondant:



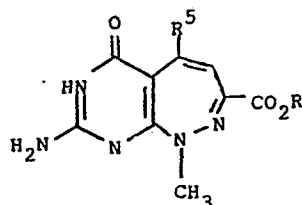
où R⁸ représente un radical alcoyle inférieur (C₁₋₄), un radical phényle (éventuellement substitué par un ou plusieurs radicaux hydroxyle ou alcoxy inférieurs) ou un radical pyridyle, qui comprend le chauffage dans un alcoylcellosolve ou le diméthylsulfoxyde d'un composé de formule (XVIII):



où R^8 est tel que défini ci-dessus.

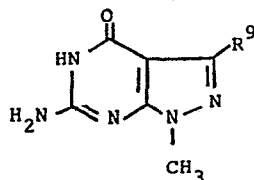
9. Composé de formule (XVIII) tel que défini dans la revendication 8.

10. Procédé de préparation d'un composé de formule (XVIII) tel que défini dans la revendication 8, qui comprend la saponification d'un composé de formule (VII):



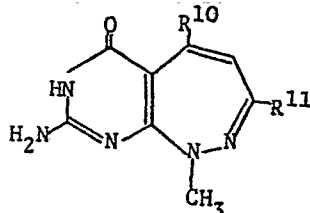
15 où R^5 est choisi entre un radical alcoyle inférieur (C_{1-4}), un radical phényle [éventuellement substitué par un ou plusieurs radicaux hydroxyle ou alcoxy inférieurs (C_{1-4})] et un radical pyridyle et R est un radical alcoyle inférieur (C_{1-4}).

11. Procédé de préparation d'un composé de formule (XIX) ou d'un tautomère ou sel correspondant:



(XIX)

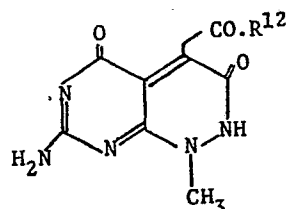
30 où R^9 est choisi entre un radical alcoyle inférieur (C_{1-4}), un radical phényle [éventuellement substitué par un ou plusieurs radicaux hydroxyle ou alcoxy inférieurs (C_{1-4})] et un radical pyridyle, qui comprend le chauffage, dans un acide aqueux, d'un composé de formule (XX):



(XX)

45 où R^{10} est choisi entre un radical alcoyle inférieur (C_{1-4}), un radical phényle [éventuellement substitué par un ou plusieurs radicaux hydroxyle ou alcoxy inférieurs (C_{1-4})] et un radical pyridyle et R^{11} est choisi entre un radical alcoyle inférieur (C_{1-4}), un radical phényle [éventuellement substitué par un ou plusieurs radicaux hydroxyle ou alcoxy inférieurs (C_{1-4})], un radical pyridyle et un radical CO_2R où R est un radical alcoyle inférieur (C_{1-4}), étant entendu que lorsque R^{11} n'est pas un radical CO_2R , R^{10} et R^{11} sont identiques.

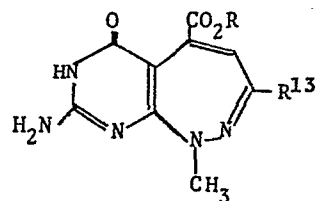
12. Procédé de préparation d'un composé de formule (XXI) ou d'un tautomère ou sel correspondant:



(XXI)

60 où R^{12} est un radical alcoyle inférieur (C_{1-4}), qui comprend le traitement d'un composé de formule (XXII) au moyen d'un acide aqueux à la température ambiante:

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(XXII)

où R et R¹³ sont tous deux des radicaux alcoyle inférieurs (C₁₋₄).