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(54) Title: PHARMACEUTICAL COMPOSITION

(57) Abstract: The present invention relates to pharmaceutical antimicrobial compositions and in particular to such compositions comprising 2,4-diamino-6-(hydroxymethyl) pteridine (DAP), 2,4-diaminopteroic acid (DAPA) and 2,4-diamino-N10-methylpteroic acid (DAMPA) and their derivatives or analogues thereof.

DESCRIPTION

PHARMACEUTICAL COMPOSITION

The present invention relates to pharmaceutical antimicrobial, for example antibacterial, compositions and in particular to such compositions comprising 2,4-diamino-6-(hydroxymethyl) pteridine (DAP), 2,4-diaminopteroic acid (DAPA) and 2,4-diamino-N10-methylpteroic acid (DAMPA) and their derivatives or analogues thereof.

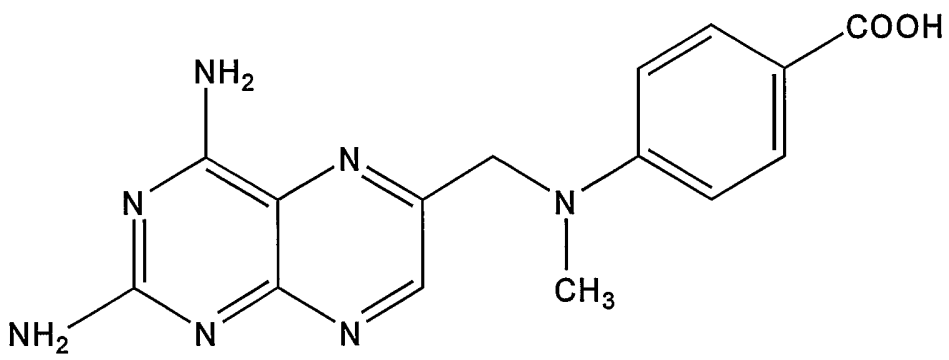
Under normal conditions in the folate biochemical pathway, dihydropteroate synthase (DHPS) condenses 2-amino-4-hydroxy-6-hydroxymethyldihydro-pteridine pyrophosphate (HMDP-PP) with para amino benzioc acid (pABA) to synthesise dihydropteroate (DHP). DHP is further glutamated by dihydrofolate synthase enzyme (DHFS) to generate dihydrofolate. Dihydrofolate (DHF) is then reduced to tetrahydrofolate by dihydrofolate reductase enzyme (DHFR) before entering the tetrahydrofolate pool.

Aminopterin (APT) and methotrexate (amethopterin, MTX) were the first antifolate drugs used against rapidly dividing (tumour) cells in human. Both APT and MTX are 2,4-diamino-pteridine compounds that compete with folate, because of their similar structure. Folate molecules comprise 2-amino-4-hydroxy-pteridine coupled to pABA and a glutamate moiety, and both APT and MTX have the 4-hydroxy replaced by 4-amino; thus, APT and MTX comprise 2, 4-diamino pteridine coupled to pABA and glutamate. MTX has, in addition, a methyl group on N (10).

Folate analogues such as trimethoprim have been found to have a potent antibacterial and antiprotozoal activity. It has also been found that trimethoprim binds 10^5-fold less tightly to mammalian DHFR than to the DHFR of susceptible microorganisms, with the small differences in the active-site clefts of these enzymes resulting in the highly selective antimicrobial action. A widely used method of treating these types of infections in humans is the combination of trimethoprim and sulfamethoxazole.

Several biochemical inhibitors (namely 2,4-diaminopteridines and 2,4-diaminoquinazolines) of the pteridine pathways of the parasite *Leishmania* have been tested and their potency assessed against antifolate-resistant mutants *(Hardy, L.W. et al., Exp. Parasitology (1997) 87, 157-169)*. Although it was concluded that potent inhibition of a novel alternative pteridine reductase which is relatively insensitive to MTX was insufficient for growth inhibition of the parasite.

DAMPA has never been described as an antimicrobial or antibacterial agent and has the structure identified below:

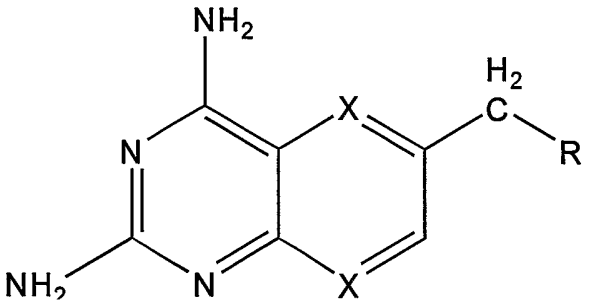


DAMPA is one of the metabolites of MTX in humans and its toxicity profile and pharmacokinetic properties have been studied in non human primates within the context of reducing the toxicity of MTX in patients with MTX-induced renal failure by the rescue agent, carboxypeptidase-G_2 *(Widemann et al., J Pharmacol Exp Ther. 2000, 294(3):894-901)*. DAMPA was found to be safe at a dose of 200 mg/m2 in Rhesus monkeys and has a short half-life of less than 1 hour.

It has been reported that both MTX and APT have Ki to *Escherisha coli* bacteria values similar to purified human dihydrofolate reductase (DHFR) (*Appleman et al. 1988, J Biol Chem, 263: 10304-13*). However, the inability of MTX and APT to cross bacteria cell walls and/or their efflux by multidrug resistance proteins makes these compounds weak inhibitors of bacterial growth (*Kopytek et al. 2000, Antimicrobial Agents Chem, 44: 3210-2*). A compound which could be used by bacteria as a folate precursor and which could be converted *de novo* to MTX or APT could be toxic to bacterial organisms. Such compounds could have lower, or no, toxicity to humans because mammalian cells lack the complete pathway leading to synthesis of folate derivatives.

According to the present invention there is provided a folate precursor, or derivative or analogue thereof, for use as an antibacterial/antimicrobial compound.

In accordance with an aspect of the present invention, there is provided an antimicrobial compound of the general formula:



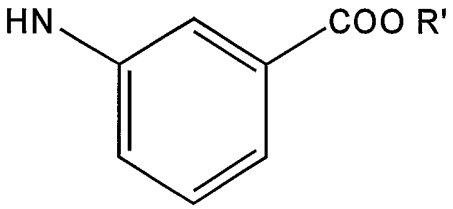
where:

X is carbon or nitrogen

R is selected from the group consisting of:

a) O - R'

b)



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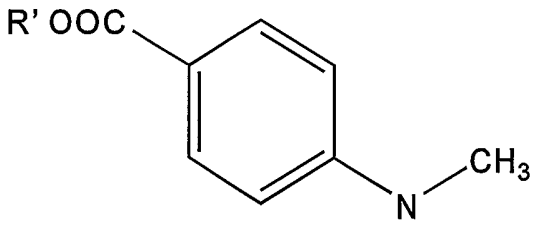

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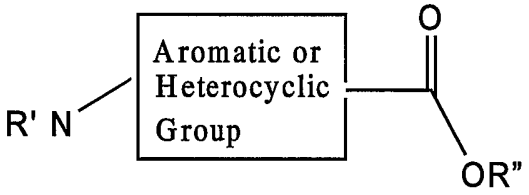

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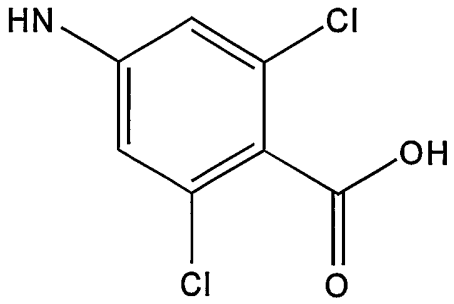

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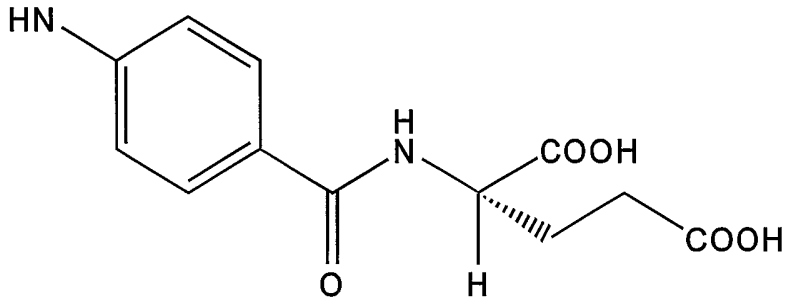
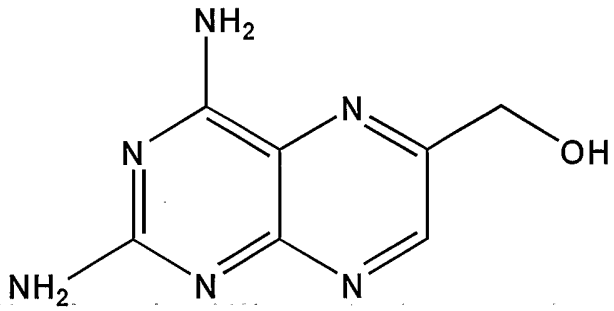


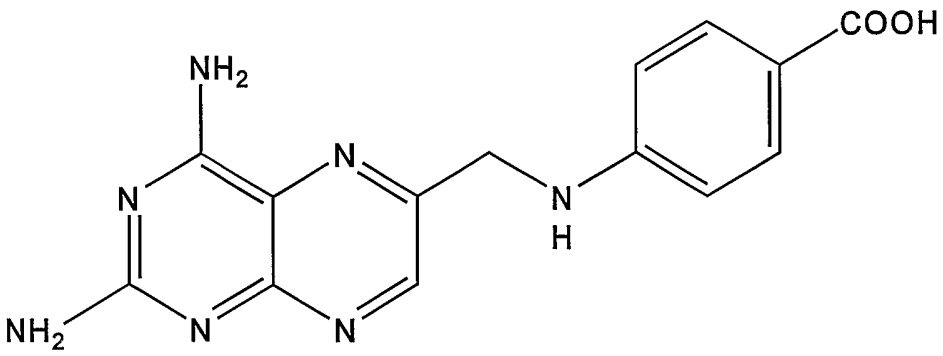
wherein R' and R" are independently selected from the group consisting of hydrogen, alkyl, aryl, aralkyl, alkaryl, cycloalkyl and alkenyl.

A preferred antimicrobial compound according to the invention is 2,4-diamino-6-(hydroxymethyl) pteridine (DAP) with the formula:



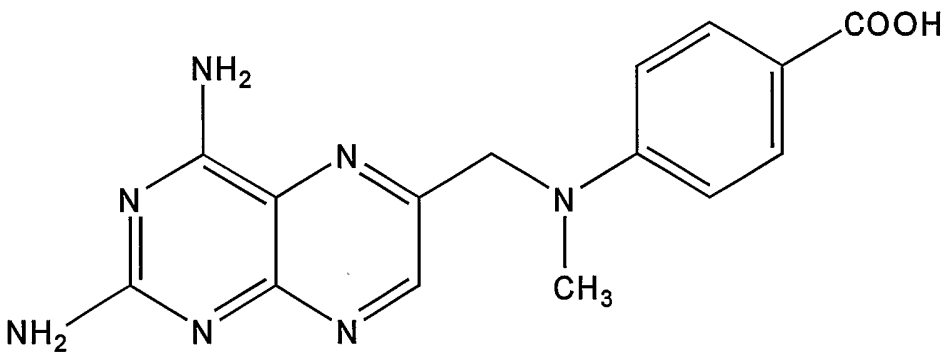
or derivative or analogue thereof.

Another preferred antimicrobial compound according to the invention is 2,4-diaminopteroic acid (DAPA) with the formula:



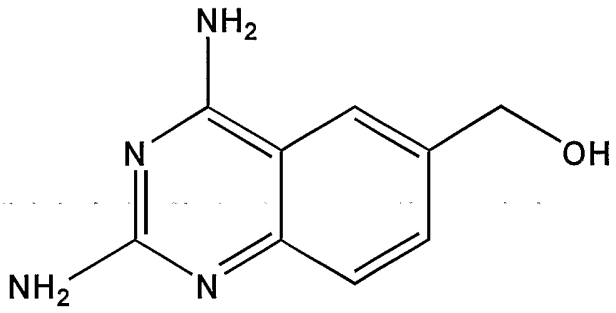
or derivative or analogue thereof.

A further preferred antimicrobial compound according to the invention is diamino-N10-methylpteroic acid (DAMPA) with the formula:



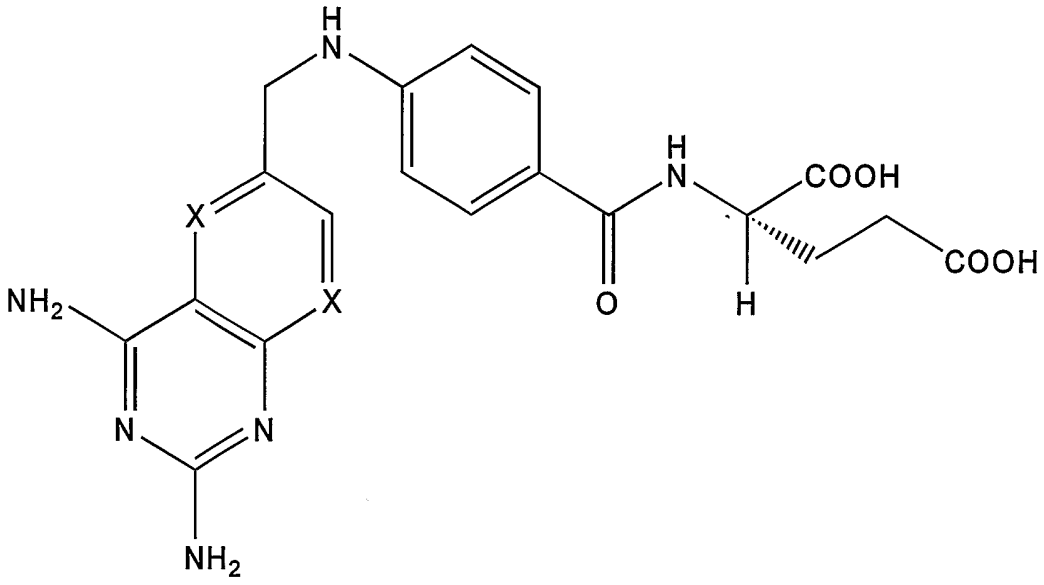
or derivative or analogue thereof.

A further preferred antimicrobial compound according to the invention has the formula:



or is a derivative or analogue thereof.

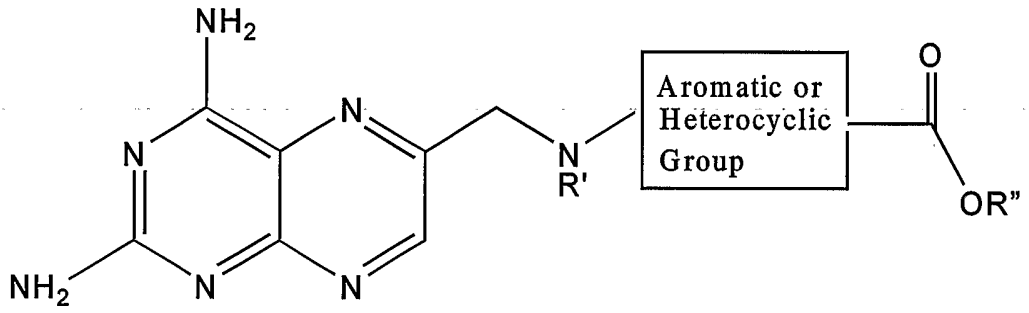
Yet another preferred group of antimicrobial compounds have the general formula:



where:

X is selected from either carbon or nitrogen.

Another preferred group of antimicrobial compounds according to the invention have the general formula:

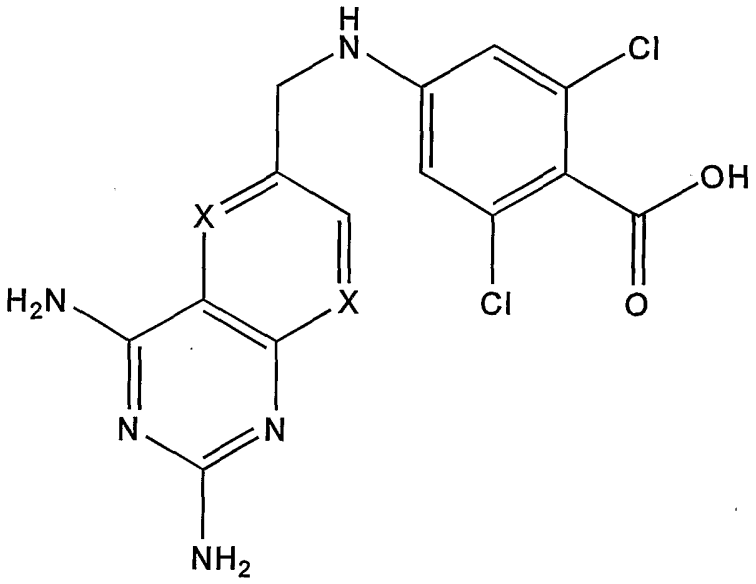


where:

R" is selected from hydrogen, alkyl, aryl and R' is selected from hydrogen or methyl.

Another preferred group of antimicrobial compounds according to the

invention have the general formula:



where:

X is selected from either nitrogen or carbon.

The compounds of the invention are preferred as antibacterial pharmaceutical agents.

In accordance with yet another aspect of the present invention, there is provided a pharmaceutical antimicrobial or antibacterial preparation comprising, as an active ingredient, a compound as herein described.

DAP, DAPA and DAMPA are widely and cheaply available compounds which have relatively low toxicity towards humans. The use of these compounds as antimicrobial or antibacterial agents may have additional benefits of not only being effective against microbial or bacterial attack, but also provide additional compounds with which to use in combination therapies with established drugs to enable and enhance their efficacy.

In an embodiment of the present invention, there is a compound as herein described, or a pharmaceutically active salt thereof, for use in the manufacture of a medicament for use as an antimicrobial or antibacterial agent.

The pharmaceutical preparation comprising, as an active ingredient, a compound as herein described may be prepared as a hydrogen chloride salt. Alternatively, the compound may be prepared as a phosphate salt.

In an embodiment of the present invention, there is provided a pharmaceutical preparation for the treatment or prophylaxis of microbial or bacterial infections comprising, as an active ingredient, a compound as herein described. The pharmaceutical preparation may be produced into a pharmaceutically active salt.

The pharmaceutical compositions according to the present invention, will now be more particularly described by way of example only.

The inventors have shown that HMDP-PP condenses with sulfa-drugs to generate HMDP-SD adducts, as is described in co-pending UK patent application no. 0211448.6 . Since the malaria parasite can metabolise sulfadoxine (SDX) and dapsone (DDS), analogs of HMDP can also serve as precursors in the folate biosynthesis pathway by the parasite. It was hypothesised that, 2, 4-diamino-4-hydroxy-6-hydroxymethyldihydro-pteridine (DAP), a HMDP analog that has the pterin ring of APT and MTX, will also be condensed with pABA to generate the DAP-pABA adduct, after its pyrophosphorylation. The glutamation of this DAP-pABA adduct by dihydrofolate synthase (DHFS) would then generate aminopterin (APT) *de novo*. This *in situ* synthesised APT should then inhibit DHFR, and

other folate enzymes downstream in pathway. Similarly, diaminopetroic acid (DPA), an analog of dihydropterioc acid, the parasite will generate APT *de novo,* glutamation by DHFS enzyme and the incubation of diamino-N-methyl-methyl hydroxy-Pteroic acid (DAMPA) will lead to the synthesis of MTX after DAMPA glutamation by DHFS. These compounds, as discussed previously, will inhibit parasite folate enzymes. The biosynthetic generation of the toxic MTX and APT should not affect the host, because the host lacks the enzymes for endogenous folate biosynthesis.

This proposed mode of action of these antimetabolites in *P. Falciparum* may also apply to other microorganisms that are reliant upon *de novo* folate synthesis. Examples of such microorganisms include other apicomplexa parasites, bacteria and fungi.

In order to establish whether DAP, DAPA and DAMPA would have antibacterial activity, an *in vitro* experiment was carried out to assess their action on reference isolates. Minimum inhibitory concentrations (MIC) of DAP, DAPA and DAMPA were determined by a standard plate incorporation method according to international standards (NCCLS, 1997, *National Committee for Clinical Laboratory Standards). Methods for dilution antimicrobial susceptibility for bacteria that grow aerobically; Approved standards 4th edn. NCCS document M7-A4, Wayne PA, USA).*

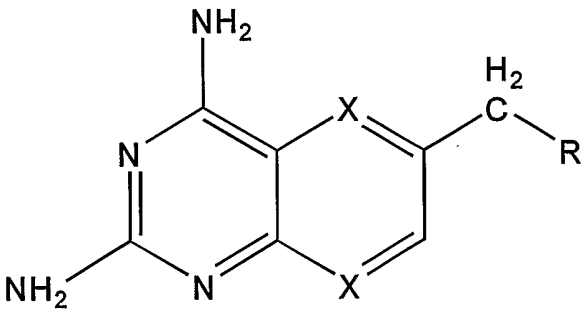
Serial doubling dilutions or doubling increases starting at 1 mg/L of the agents were incorporated into appropriate agar growth media. A wide selection of microorganisms were individually suspended in broth to a concentration that when

delivered by a mulitpoint innoculator would result in ca. 105 bacteria per spot. After inoculation, the plates were incubated for 18 hours in air or air with 10% CO_2 as appropriate at 37°C. Presence or absence of growth was recorded. The MIC was taken as the lowest concentration of the agent that restricted the bacterial growth to five or fewer colonies.

The data showed that these compounds bear antibacterial activity at concentrations well below those likely to be achievable in plasma against *Streptoccoccus pyogenes* (group A strep), Group B streptococci, *Streptococcus viridans* (all with MICs of 1 mg per litre or less), *Moraxella catarrhalis* (MIC 4 mg per litre). In addition, DAP, DAPA and DAMPA show promising activities against *Haemophilus influenzae, Neisseria meningitidis* and *Neisseria gonorrhoeae*. This indicates that one or more of the compounds may have useful activity in treating for example, bacterial meningitis and exacerbation of infection in chronic obstructive airway disease. In addition, these compounds could be used as leads for the synthesis of analogs that can be potent against other bacteria species.

CLAIMS

1. An antimicrobial compound of the general formula:



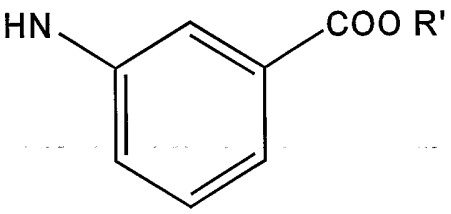
where:

X is carbon or nitrogen

R is selected from the group consisting of:

a) O - R'

b)



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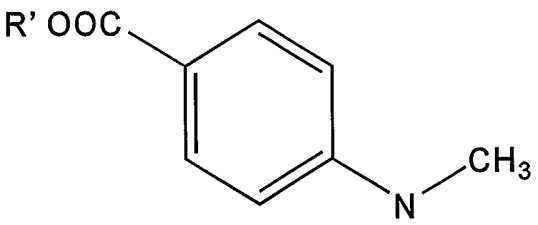

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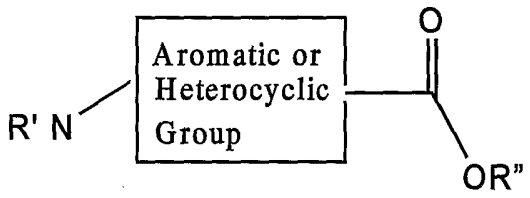

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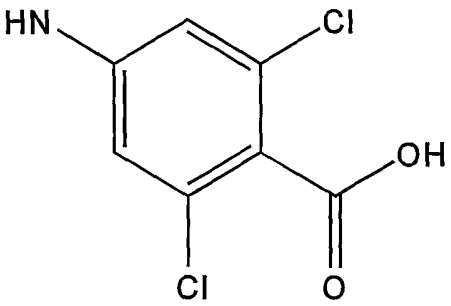
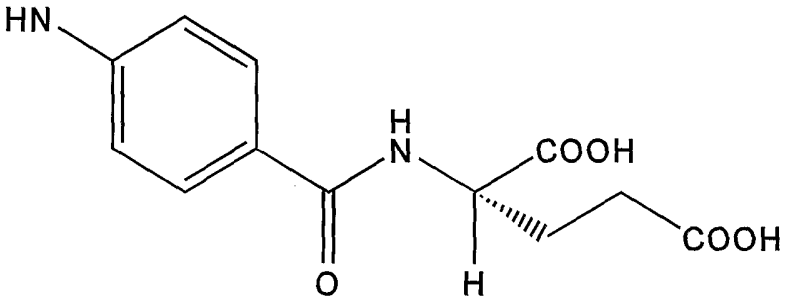
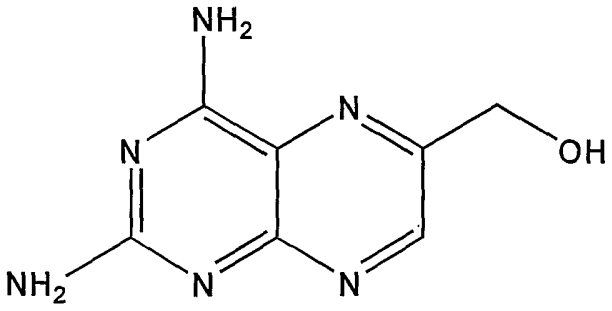


f)



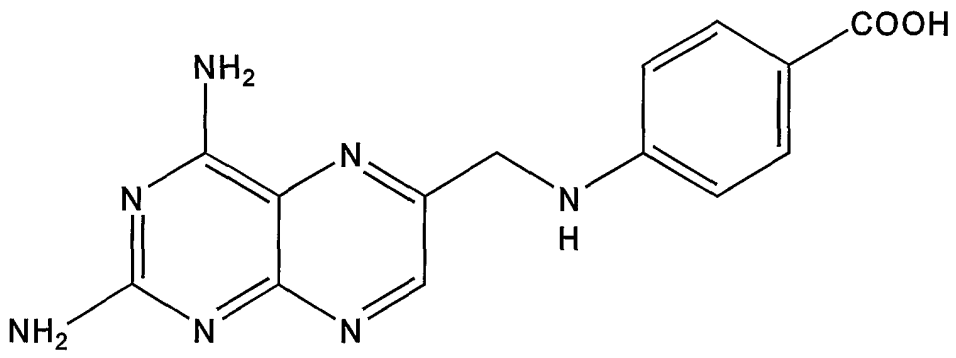
wherein R' and R" are independently selected from the group consisting of hydrogen, alkyl, aryl, aralkyl, alkaryl, cycloalkyl and alkenyl.

2. An antimicrobial compound as claimed in claim 1, wherein the compound is 2,4-diamino-6-(hydroxymethyl) pteridine (DAP) with the formula:



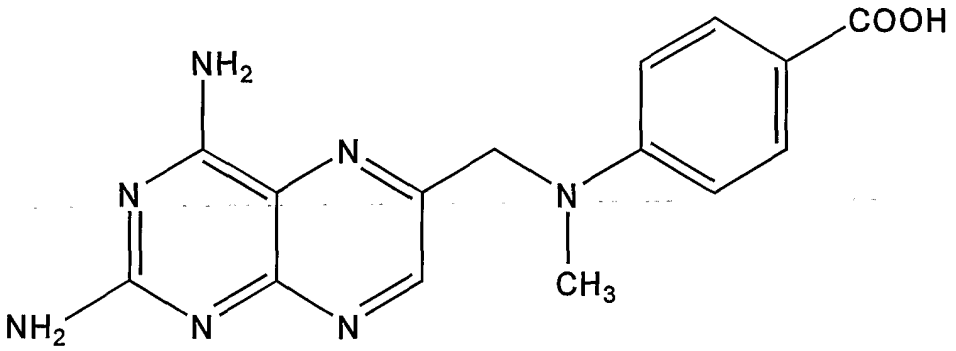
or derivative or analogue thereof.

3. An antimicrobial compound as claimed in claim 1, wherein the compound is 2,4-diaminopteroic acid (DAPA) with the formula:



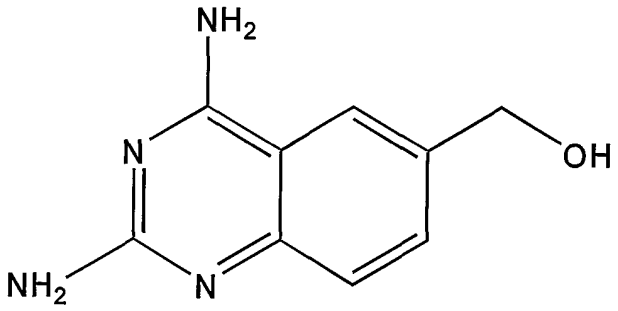
or derivative or analogue thereof.

4. An antimicrobial compound as claimed in claim 1, wherein the compound is diamino-N10-methylpteroic acid (DAMPA) with the formula:



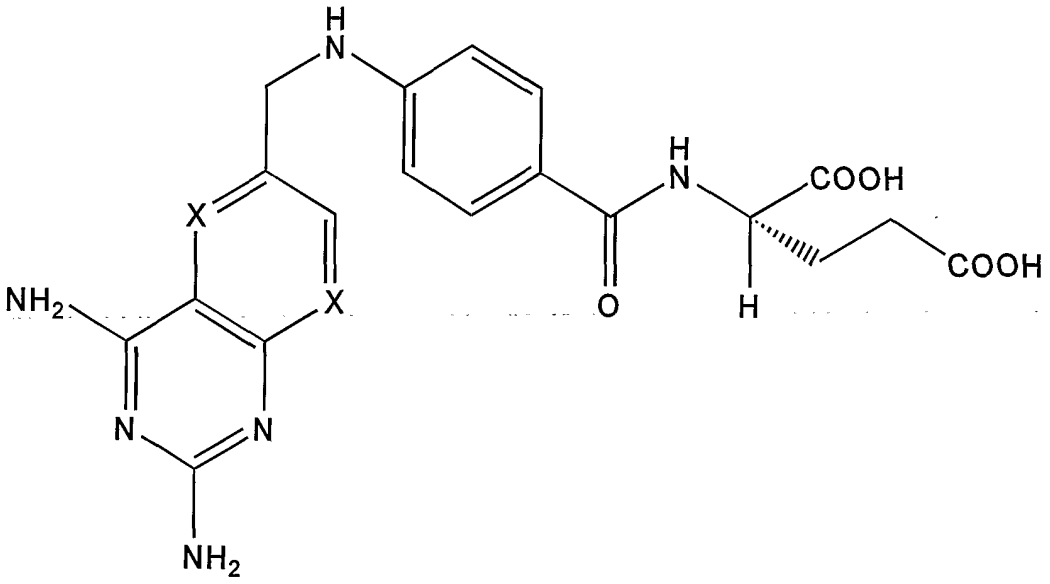
or derivative or analogue thereof.

5. An antimicrobial compound as claimed in claim 1, wherein the compound has the formula:



or is a derivative or analogue thereof.

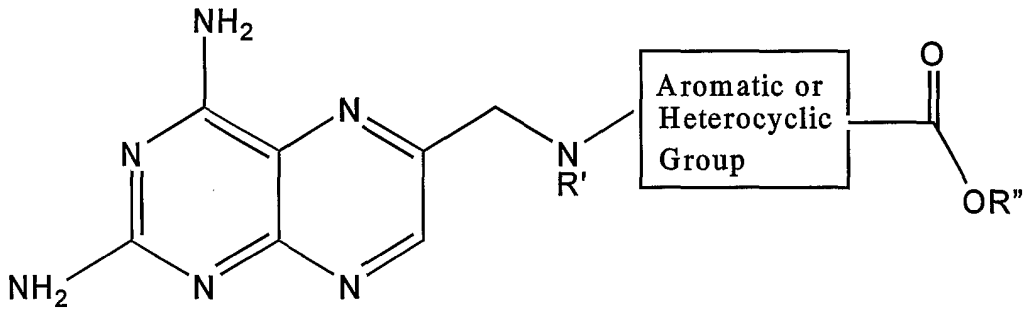
6. An antimicrobial compound as claimed in claim 1, wherein the compound has the formula:



where:

X is selected from either carbon or nitrogen.

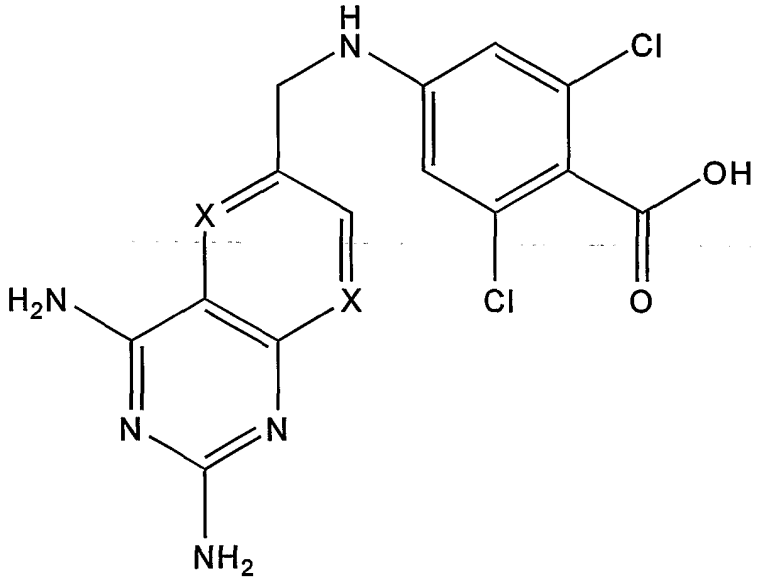
7. An antimicrobial compound as claimed in claim 1, wherein the compound has the formula:



where:

R" is selected from hydrogen, alkyl, aryl and R' is selected from hydrogen or methyl.

8. An antimicrobial compound as claimed in claim 1, wherein the compound has the formula:



where:

X is selected from either nitrogen or carbon.

9. A compound as claimed in any one or claims 1 to 8, wherein the compound is used as an active ingredient in a pharmaceutical antimicrobial or antibacterial preparation.

10. A compound as claimed in any one of claims 1 to 9, wherein the compound or a pharmaceutically active salt thereof is used in the manufacture of a medicament for use as an antimicrobial or antibacterial agent.

11. A compound as claimed in any one of claims 1 to 10, wherein the compound is prepared as a hydrogen chloride salt.

12. A compound as claimed in any one of claims 1 to 10, wherein the compound is prepared as a phosphate salt.

13. A pharmaceutical preparation incorporating a compound as claimed in any one of claims 1 to 12, wherein the pharmaceutical preparation is for the treatment or prophylaxis of microbial or bacterial infections.