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NITROTHIAZOLE-HYDROXYQUINOLINE COMPOSITIONS

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The present invention relates to nitrothiazole-hydroxyquinoline compounds, and more particularly to compositions made up of a 5-nitro-2-thiazolyl derivative on the one hand, and 8-hydroxyquinoline or a derivative thereof on the other.

It has been found that in the treatment of certain protozoan infections as hereinafter set forth, these two types of materials when applied together as a single medicament exert an unexpected synergistic action, making the combination thereof unexpectedly more effective than is either of the two active ingredients thereof alone or any reasonably-to-be-expected result of the additive action of the two. This synergistic action is exerted against certain types of trichomonal organisms which make the compositions of the present invention unusually advantageous for the treatment of certain gynecologic ailments.

Leukorrhea or an abnormal discharge from the female genital tract is one of the chief gynecologic complaints. It may arise from a number of causes. One of the most common and difficult to treat is an infection with *Trichomonas vaginalis*. Various agents have been devised to help manage this problem. Those in common use may be divided into groups according to the active principle as follows:

(1) Arsenic-containing chemicals
(2) Mercury-containing chemicals
(3) Hydroxyquinoline or similar compounds
(4) Sulfonamides
(5) Halogens
(6) Antibiotics
(7) Nitrofurans
(8) Nitrothiazoles
(9) Detergents
(10) Chelating agents

Tests frequently show the presence of more than one organism, which may be a factor complicating successful therapy. Thus, it may be necessary to use two or more antimicrobiological drugs to eradicate the infection. While many combinations of ingredients have been tried on an empiric basis in an endeavor to produce more effective medication, the results have been less than anticipated and the medical profession is most anxious to have available better methods for control of this problem. Thus far, combinations have demonstrated at the best only additive effects.

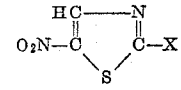
The accepted method of treating trichomonal infection is by the topical application of a trichomonacide by suppository, vaginal tablet, jelly or insufflation. Acidification of the vaginal tract by vinegar douches is common adjunctive therapy.

It is already known that 8-hydroxyquinoline and halogenated 8-hydroxyquinolines are inhibitory toward certain bacteria, fungi, and protozoa. Also, the substituted 5-nitro-2-thiazolyl piperazines disclosed in a copending application of Reisner et al., Serial No. 2.137, filed January 13, 1960, now Patent No. 3,021,333, issued February 13, 1962, are inhibitory toward protozoa, particularly *Trichomonas vaginalis* and *Trichomonas foetus*. In addition, certain other 5-nitro-2-thiazolyl derivatives are known to be effective against these organisms. Examples of these additional compounds will be given below.

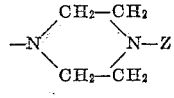
The present invention is based upon the discovery of the novel synergistic effects resulting from the use of 8-hydroxyquinolines and nitrothiazole compositions wherein the inhibitory power with respect to the organisms named is found to be very much larger than the sum of the activities of the individual compounds when used separately.

For the purposes of the present invention, one of the two essential ingredients is a material selected from the group consisting of 8-hydroxyquinoline and 5-chloro-7-iodo-8-hydroxyquinoline.

The other essential ingredient in accordance with the present invention is a nitrothiazole derivative having the formula:



wherein X is selected from the group consisting of NH_2, $NH\cdot CO\cdot CH_3$, $NH\cdot CO\cdot NH\cdot C_2H_5$, and



in which Z is selected from the group consisting of H; lower alkyl; hydroxy-lower-alkyl; carbalkoxy; acyl derived from alkanoic, haloalkanoic, aralkanoic and aryl acids; formyl; and formamido.

Compounds and compositions according to the present invention have been used clinically on human beings afflicted with the types of infections hereinabove referred to and have proved themselves to be superior to the same active ingredients when used separately. Confirming these tests, mathematical data proving the synergistic activity of compositions in accordance with the present invention has been obtained from a series of in vitro tests as follows:

Trichomonas vaginalis, strain 1 Kupferberg, was grown in simplified trypticase serum broth, pH 6.0. *Trichomonas foetus*, strain BR, was grown in simplified trypticase serum broth, pH 7.0. Trichomonal cultures were incubated at 37° C. for 48 hours after which time they reached a cell density of 1 to 3×10^6 cells per ml. Graded amounts of the drug under test were added to tubes of S.T.S. to give final concentrations of from 1000 p.p.m. to 0.5 p.p.m. To each of these tubes was added 0.5 ml. from the 48-hour seed cultures. Drug-free solvent controls (propylene glycol for the nitrothiazoles, ethanol for 8-hydroxyquinoline or iodochlorohydroxyquinoline) were similarly inoculated. All tubes were incubated at 37° C. for 48 hours; the presence or absence of trichomonads was then determined microscopically.

The following nitrothiazolyl piperazine derivatives were tested in this way, the symbol "Z" in each instance referring to the formula hereinabove given.

(I) Z=hydrogen
(II) $Z=-\overset{\overset{O}{\|}}{C}-N(C_2H_5)_2$
(III) $Z=-CH_2\cdot CH_2\cdot OH$
(IV) $Z=-CH_2\cdot(CH_2)_5\cdot CH_3$
(V) $Z=-\overset{\overset{O}{\|}}{C}-O\,C_2H_5$
(VI) Z=—CHO
(VII) $Z=-\overset{\overset{O}{\|}}{C}-CHCl_2$

(VIII) $Z{=}\!-\!\overset{\overset{O}{\|}}{C}\!-\!CH\cdot(CH_3)_2$

(IX) $Z{=}\!-\!\overset{\overset{O}{\|}}{C}\!-\!CH_3$

(X) $Z{=}\!-\!\overset{\overset{O}{\|}}{C}\!-\!CH_2\!-\!C_6H_5$

(XI) $Z{=}\!-\!\overset{\overset{O}{\|}}{C}\!-\!CH_2\!-\!CH_2\!-\!CH_3$

(XII) $Z{=}\!-\!\overset{\overset{O}{\|}}{C}\!-\!C_6H_5$

In addition, a number of amino and substituted aminothiazole compounds, which were known to have some independent activity in the treatment of trichomonal infections as aforesaid were similarly tested, the symbol "X" in the formulas given below referring to the corresponding symbol in the general nitrothiazole formula given above.

(XIII) $X{=}\!-\!NH\!-\!\overset{\overset{O}{\|}}{C}\!-\!CH_3$

(XIV) $X{=}\!-\!NH\!-\!\overset{\overset{O}{\|}}{C}\!-\!NH\!-\!C_2H_5$

(XV) $X{=}\!-\!NH_2$

A series of tests were run as aforesaid, using the organism *Trichomonas foetus* in each instance. It was found that with this organism, the concentration of 8-hydroxyquinoline, when used alone, required to inhibit the organism was 125 p.p.m. (part per million). There is given in Table I which follows the inhibiting concentration of the respective nitrothiazole derivatives, when used alone, which is required to inhibit the infection-causing organism (*Trichomonas foetus*). In the tests for synergism, a concentration of 0.5 p.p.m. of each of the respective nitrothiazole derivatives was used for each test and the concentration of 8-hydroxyquinoline required to be added to this concentration of the nitrothiazole derivative was then determined. The data resulting from this series of tests is set out in Table I, which follows.

Nitrothiazole Derivative No.	Inhibiting Concentration, Nitrothiazole Derivative When Used Alone, p.p.m.	Required Concentration of 8-hydroxyquinoline+0.5 p.p.m. Nitrothiazole Derivative Tested, p.p.m.
I	1.95	3.9
II	15.6	3.9
III	7.8	1.95
IV	7.8	3.9
V	15.6	7.8
VI	15.6	15.6
VII	15.6	3.9
VIII	15.6	3.9
IX	1.95	15.6
X	7.8	3.9
XI	3.9	1.95
XII	7.8	3.9
XIII	1.95	50
XIV	1.95	31
XV	3.9	16

From Table I above it is evident that the concentrations of the nitrothiazole derivatives, when used alone, which is respectively necessary to inhibit the microorganism, ranged from slightly less than 2 p.p.m. to more than 15 p.p.m. Thus, in no instance was the 0.5 p.p.m. concentration of any of the compounds tested sufficient to inhibit the microorganism. Furthermore, while 8-hydroxyquinoline, when used alone, inhibits this microorganism only at a concentration of at least 125 p.p.m., it is noted from Table I that the addition of 0.5 p.p.m. of any of the nitrothiazole derivatives, coupled with less than half the 125 p.p.m. concentration of the 8-hydroxyquinoline, results in effective inhibition of the microorganism.

When the microorganism used for the test is *Trichomonas vaginalis* and when various of the same nitrothiazole derivative compounds are used in conjunction with both 8-hydroxyquinoline and the halogen-substituted 8-hydroxyquinoline above-identified, similar results are obtained. In this instance each of compounds IV, XIII, XIV and XV given above was tested as aforesaid in vitro against this microorganism, prepared as aforesaid. The nitrothiazole derivatives used in this series of tests were first tested alone in a concentration of 0.5 p.p.m. and were found in each instance not to inhibit the microorganism. This concentration (0.5 p.p.m.) of each nitrothiazole derivative was used respectively as in a series of tests in conjunction with various amounts of the 8-hydroxyquinoline or the halo derivative thereof above-identified. Prior to the tests for synergism using both drugs, it was found that the amount of 8-hydroxyquinoline, when used alone, which was required to inhibit this microorganism was 75 p.p.m.; and the amount of 5-chloro-7-iodo-8-hydroxyquinoline, when used alone, required to inhibit this microorganism was more than 1000 p.p.m. The results of testing the several nitrothiazole derivatives in conjunction with one or the other of the hydroxyquinoline compounds is shown in Table II, which follows:

Nitrothiazole Compound Used (at concentration of 0.5 p.p.m.)	Minimum Inhibiting Concentration of 8-hydroxyquinoline or its Derivative Combined Therewith
IX	6.3 p.p.m. 8-hydroxyquinoline.
IX	250 p.p.m. 5-chloro-7-iodo-8-hydroxyquinoline.
XIII	3.1 p.p.m. 8-hydroxyquinoline.
XIII	500 p.p.m. 5-chloro-7-iodo-8-hydroxyquinoline.
XIV	1.0 p.p.m. 8-hydroxyquinoline.
XV	31.3 p.p.m. 8-hydroxyquinoline.

From the above it is evident that synergistic activity is also present in connection with this microorganism as to all the combinations tested. Here again, combinations consisting of less than half the minimum effective concentration of each of the two active ingredients are found effective to inhibit the microorganism.

From the tests aforesaid, it is believed reasonable to assume with respect to each of the compounds tested that there are also many other substantially similar compounds having independent inhibitory characteristics as to these microorganisms, wherein the combinations will have synergistic activity when used as set forth herein.

These in vitro results were confirmed clinically with the combination of 8-hydroxyquinoline and compound No. IX. In a series of cases of infection by various species of Trichomonas the combination of these two compounds was found to be very effective with respect to rapidity of action and decreased rate of recurrence. The results obtained were believed to be far superior to any that could be obtained with either of the compounds alone, regardless of concentration used.

This composition as it is used can be in the form either of a jelly, a cream, or in some cases a suppository-type tablet. In each instance the composition consists, not only of the two active ingredients in a proportion found to be efficacious in use by clinical tests, but also of a suitable base material or carrier of an appropriate nature in view of the class of composition to be prepared and which is therapeutically acceptable in use, bland and non-irritating. Additional materials in small amounts can also be employed for controlling the pH of the composition, so as to bring the pH value slightly on the acid

side of neutral. Examples of suitable compositions of three types are as follows:

Example I.—Vaginal Cream

The base material used in this example is an oil-in-water emulsion, with which the active ingredients are present in an amount aggregating not over about 10% of the total weight. The particular ingredients given in one specific composition which has been prepared is typical of a petrolatum (as an example of an oil-in-water type) emulsion usable for this purpose.

Ingredient:	Percent by weight
Polyoxyethylene sorbitan monooleate ("Tween 80")	3.0
Stearyl alcohol	5.0
Glyceryl monostearate	15.0
Stearic acid	5.0
White petrolatum	10.0
Glycerine	15.0
Citric acid	0.25
Benzoic acid	0.15
1-acetyl-4-(5-nitro-2-thiazolyl) piperazine	1.0
8-hydroxyquinoline	0.25
Distilled water to make 100%	45.35

Example 2.—Vaginal Jelly

The base material or carrier in this instance is a suitable jelly, made from a jelling agent such as methyl cellulose, starch, vegetable gums and the like, and water, this jelly-type base being combined with the active ingredients and with minor amounts of pH controlling ingredients in the formulation of the composition as a whole. Further, in the composition which is specifically given hereinafter, a substantial amount of glycerine is used as a water-miscible solvent material assisting in the formulation of a jelly of a desired consistency as a carrier or base for the active ingredients.

Ingredient:	Percent by weight
Methyl cellulose (400 cps.)	7.5
Glycerine	15.0
Citric acid	0.1
Benzoic acid	0.1
Sodium citrate	0.2
1-acetyl-4-(5-nitro-2-thiazolyl) piperazine	1.0
8-hydroxyquinoline	0.25
Distilled water to make 100%	75.85

Example 3.—Vaginal Tablet

In this instance a suitable base or carrier for the formulation of dry solid tablets is used, preferably consisting principally of lactose, with relatively smaller amounts of starch and magnesium stearate, the latter serving, in part at least, as a lubricant in connection with the forming of the tablets of this composition. Other materials substantially equivalent to lactose, including, for example, solid, reasonably water-soluble sugars or sugar derivatives, could be used in substitution for some or all of the lactose and/or starch.

Ingredient:	Percent by weight
Lactose	85.0
Sodium citrate	0.1
Citric acid	0.4
Magnesium stearate	1.0
1-acetyl-4-(5-nitro-2-thiazolyl) piperazine	5.0
8-hydroxyquinoline	1.25
Starch	7.25

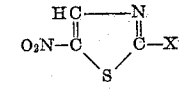
From a broad point of view it is necessary that each of the two principal ingredients of the composition shall be present in a substantial amount, which amount may be half or less that concentration which would be required if the compound in question were to be used alone as proven by the in vitro tests hereinabove set forth. In the actual examples of complete compositions hereinabove given it is noted that the nitrothiazolyl piperazine compound is used in a weight percent, which is about four times that of the hydroxyquinoline compound. This proportion is not intended as limiting upon the ratio in which these two active ingredients may be used with respect to each other, but rather such ratio is quite wide in range. In practice it is usually between about 1:10 to about 10:1, with the first figure in each ratio representing the 8-hydroxyquinoline compound and the last figure in each ratio representing the nitrothiazole derivative. It will be found that as to some of these compounds a greater ratio is more efficacious than with others. In most practical compositions a greater amount of the nitrothiazole derivative ingredient than of the hydroxyquinoline is used, as in general the former is the more active and the less irritating of these two ingredients. In any event the actual concentration of these two active ingredients in a practically useful and effective composition is many times greater than the concentrations used in the in vitro tests above set out, as shown by the actual useful compositions given above by way of examples. However, this goes not to the matter of operability or utility, but rather to a question of judgment as to the desired proportion to be used, synergistic activity being present over a wide variety of proportions or ratios.

Actual clinical tests of the compositions of the present invention have been effected, in most instances, with one or another of the compositions above given, at least as to the ratio of concentration of the two active ingredients and have used the specific active ingredients set out in these particular compositions, i.e. 8-hydroxyquinoline and 1-acetyl-4-(5-nitro-2-thiazolyl) piperazine.

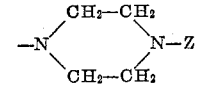
While it has been attempted throughout the foregoing description to set out the principles and the general limits of the invention as well as to give a considerable number of specific examples, other variations will suggest themselves to those skilled in the art from the foregoing particular description. We do not wish to be limited, therefore, except by the scope of the appended claims, which are to be construed validly as broadly as the state of the art permits.

What is claimed is:

1. A composition consisting essentially of (A) a material selected from the group consisting of 8-hydroxyquinoline and 5-chloro-7-iodo-8-hydroxyquinoline, and (B) a nitrothiazole derivative having the formula:



wherein X is selected from the group consisting of NH_2, $NH \cdot CO \cdot CH_3$, $NH \cdot CO \cdot NH \cdot C_2H_5$, and

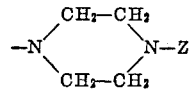


in which Z is selected from the group consisting of H; lower alkyl; hydroxy-lower-alkyl; carbalkoxy; acyl derived from alkanoic, haloalkanoic, aralkanoic and aryl acids; formyl; and formamido; said materials (A) and (B) each being present in the composition in a substantial amount.

2. The composition of claim 1, in which the weight ratio of said material (A) to said material (B) is in the range of about 1:10 to 10:1.

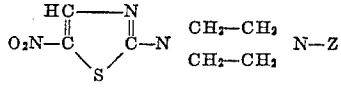
3. The composition of claim 1, in which said material (A) is 8-hydroxyquinoline.

4. The composition of claim 1, in which said material (A) is 8-hydroxyquinoline; in which X is



and in which Z is as defined in claim 1.

5. A composition consisting essentially of (A) 8-hydroxyquinoline and (B) a compound having the formula:



in which Z is lower alkanoic acyl; said materials (A) and (B) each being present in the composition in a substantial amount.

6. A composition useful for the treatment of trichomonal infections, the essential active ingredients of which consist of 8-hydroxyquinoline and 1-acetyl-4-(5-nitro-2-thiazolyl) piperazine.

7. A composition in accordance with claim 6, in which the ratio of said two essential ingredients to each other is from about 1:10 to about 10:1.

8. The composition consisting essentially of 8-hydroxyquinoline and 1-acetyl-4-(5-nitro-2-thiazolyl) piperazine in which these materials are present in the proportion of about one part by weight of the 8-hydroxyquinoline to four parts by weight of the piperazine compound aforesaid.

9. A vaginal cream, consisting essentially of 8-hydroxyquinoline and 1-acetyl-4-(5-nitro-2-thiazolyl) piperazine as essential active ingredients; and an oil-in-water emulsion carrier base, which is therapeutically acceptable, bland and non-irritating.

10. A vaginal cream, consisting essentially of the following materials in the proportion by weight given:

Ingredients:	Percent by weight
Polyoxyethylene sorbitan monooleate	3.0
Stearyl alcohol	5.0
Glyceryl monostearate	15.0
Stearic acid	5.0
White petrolatum	10.0
Glycerine	15.0
Citric acid	0.25
Benzoic acid	0.15
1-acetyl-4-(5-nitro-2-thiazolyl) piperazine	1.0
8-hydroxyquinoline	0.25
Distilled water to make 100%	45.35

11. A vaginal jelly, consisting essentially of 8-hydroxyquinoline and 1-acetyl-4-(5-nitro-2-thiazolyl) piperazine as essential active ingredients; and an aqueous-jelly carrier base, which is therapeutically acceptable, bland and non-irritating.

12. A vaginal jelly, consisting essentially of the following materials in the proportion by weight given:

Ingredients:	Percent by weight
Methyl cellulose (400 cps.)	7.5
Glycerine	15.0
Citric acid	0.1
Benzoic acid	0.1
Sodium citrate	0.2
1-acetyl-4-(5-nitro-2-thiazolyl) piperazine	1.0
8-hydroxyquinoline	0.25
Distilled water to make 100%	75.85

13. A vaginal tablet consisting essentially of 8-hydroxyquinoline and 1-acetyl-4-(5-nitro-2-thiazolyl) piperazine as essential active ingredients; and a solid tablet-forming carrier base, which is substantially water-soluble, therapeutically acceptable, bland and non-irritating.

14. A vaginal tablet, consisting essentially of the following materials in the proportion by weight given:

Ingredient:	Percent by weight
Lactose	85.0
Sodium citrate	0.1
Citric acid	0.4
Magnesium stearate	1.0
1-acetyl-4-(5-nitro-2-thiazolyl) piperazine	5.0
8-hydroxyquinoline	1.25
Starch	7.25

15. A composition in accordance with claim 1, in which the material (A) is 8-hydroxyquinoline, and in which the material (B) is one in which X is —NH·CO·CH_3.

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