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ANTIMICROBIAL ACTIVITY OF NOVEL SYNTHESIZED FUSED 6-AMINO-2-THIOURACILS

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ABSTRACT.

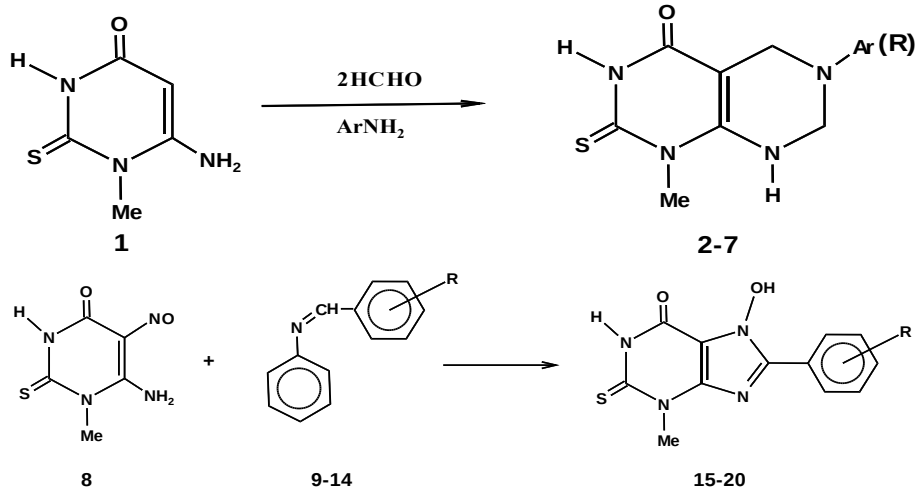
Two groups of previously synthesized thiopurine derivatives were subjected to study its antimicrobial activity against both Gram-positive, Gram –negative bacteria, yeast and fungi. The first group of the synthesized compounds, 1-methyl-6-substituted-2, 5, 7, 8 tetrahydro-2-thio-4-oxo-pyrimido [4, 5-d] pyrimidines (**2-7**) and the second group, 8-substituted-3-methyl-7-hydroxy-2-thioxanthines (**15-20**) were used. The synthesized compounds showed various inhibitory effects against Gram-positive and Gram negative bacteria as well as fungi while Yeast showed resistance towards all of the synthesized compounds. It was clear that the inhibition activity depends up on the type of substitution.

Keywords: Antimicrobial activity**,** 1-methyl-6-substituted-2,5,7,8 tetrahydro-2-thio-4-oxo-pyrimido[4,5-d] pyrimidines and 8-substituted-3-methyl-7-hydroxy -2-thioxanthines.

INTRODUCTION

Antimetabolites of purine and pyrimidine are of great interest because they may incorporate into DNA and RNA of tumour cells and as such may even inhibit the synthesis of these polynucleotides (Weber *et al* 1980, Natsumeda *et al* 1984 and Marijnen *et al* 1989). Modified purines bearing substituents at the 2-, 6- and/or 9- positions have been associated with a wide variety of interesting biological properties. For instance, they find application as cyclin-dependent kinase inhibitors (Legraverend *et al* 1998 and Chang *et al* 1999). Adenosine receptor antagonists (Wanner *et al* 2000 and Abiru *et al* 1992), modulators of multidrug resistance (Dhainaut *et al* 1996). Many derivatives of 2- thiouracils have been prepared and tested for physiological activity (Wamhoff *et al* 1992, Nogimori *et al* 1985 Bywater *et al* 1945 and Nagamatsu *et al* 1992). Amongst a variety of compounds known to interfere with metabolism in proliferating cell, antagonists of nucleic acids and their constituents have gained special significance since some of them exhibit considerable cytotoxic and antiviral activities(Youssif *et al* 1999). In particular, sulphur-containing analogous of purine base, such as 6-mercabtopurine and its derivatives have been widely used as drugs in cancer chemotherapy. Thiopurines has antiviral effects against herpes simplex virus (Campisi and Pardee 1981), influenza viruses (Yamamoto *et al* 1988), and SV40 (Maybaum *et al* 1987). These therapeutic agents are transformed through cellular processing of the active metabolite 6-thioguanine triphosphate, and then incorporated into the DNA duplex (Elion 1989 and Lepage 1963). Due to the importance of N-substituted 6-amino-2-thiouracils with respect to their antitumor activity and in extension to our work (Youssif *et al* 2008) we look to study the antimicrobial activity of newly synthesized fused 6-amino-2-thiouracils. Twelve compounds were synthesized by Youssif (2008), these compounds related to two groups, the first group (**2-7**) are, 1-methyl-6-

substituted-2,5,7,8 tetrahydro-2-thio-4-oxo-pyrimido[4,5-d] pyrimidines. The second group (**15-20**), 8-substituted-3-methyl-7-hydroxy-2-thioxanthines.The structure of these compounds is shown in Scheme 1.



Scheme 1

MATERIAL AND METHODS

The newly synthesized compounds have been tested for their antimicrobial activity against some microorganisms. The following microorganisms have been selected.

1- Bacteria, a) Gram positive bacteria; *Bacillus subtilis*, b) Gram negative bacteria; *Escherichia coli*
2- Yeast; *Candida tropicalis* (NRC)
3- Fungi; *Aspergillus niger* (NRC)

All bacterial, yeast and fungal strains were obtained from Microbial Biotechnology department (NRC); Egypt.

Antimicrobial (bacteria, yeast and fungi) Bioassay Growth media

a) Nutrient Agar Medium for Bacteria (Bridson, 1998)

The medium prepared by dissolving the following ingredients (g/L) in distilled water and autoclaved for 15 min, beef extract (1 g), yeast extract (2 g), peptone (5 g), sodium chloride (5 g), agar (20 g) and, Tween -60 (10 cm^3). pH was adjusted to 7.4.

b) Malt Extract Agar Medium for Yeast and Fungi (Bridson 1998)

The medium consisted of the following ingredients (g/L): malt extract (3 g), peptone (5 g), agar (20 g) and Tween -60 (10 cm^3); the *pH* was adjusted to 5.4.

Broth Media

For preparation of the inoculum of each microorganism the broth of both media was prepared with the same method but without agar.

Modified Agar Diffusion Cylinder Method (Bauer *et al* 1966)

1) Preparation of inoculum

The broth media were used for about 18-20 h till the optical density (O.D) became in the range 0.2-0.5 for bacteria and yeast. The broth media were used for about 48 h for fungi. The mycelial fragments were removed and the solution was used for inoculation for fungi.

2) Preparation of plates and inoculation

0.5 cm^3 of inoculums was added to 24 cm^3 of agar malted media (50^oC) and mixed by simple inversion. The malted media were poured into each 150 mm sterile Petri plate. Cut 4-6 mm wells in the harden seeded plates by Pasteur pipette and then wells were filled to surface with the various compounds (stock solution, 5 mg / cm^3 DMSO). The plates were incubated at 37^oC for 24 h for bacteria and yeast and for 48-72 h for fungi. The diameter of the inhibition zones (mm) was measured and recorded.

Determination of the minimum inhibitory concentration (MIC)

Serial dilutions of the promising compounds were subjected to *MIC* determination, it was prepared in the following pattern; 400 μg / cm^3, 200 μg / cm^3, 100 μg / cm^3, 50 μg / cm^3, 10 μg / cm^3 and, 5 μg / cm^3. *Bacillus subtilis* was selected to be the test organism where it was the most sensitive organism. The different concentrations of each compound were tested with the Modified Agar Diffusion Cylinder method as was described before.

RESULTS AND DISCUSSION

All the synthesized compounds were tested against bacteria both Gram– positive and Gram–negative bacteria, yeast and fungi. The results are shown in Table (1). The first group of the synthesized compounds **2-7,** showed a good inhibitory effect against Gram–positive bacteria and a moderate activity against Gram-negative bacteria, the activity depends on the substitution in the position no 6. When the substitution was with phenyl group,compound **13**, 1-methyl-6-phenyl-2,5,7,8 tetrahydro-2-thio-pyrimido [4,5-d] pyrimidine 4-one, showed a moderate antimicrobial activity against both gram positive and Gram –negative as well as *A. niger* and has no inhibitory effect on yeast. At the same time when the substitution was with substituted phenyl group as 1-methyl-6-(4 –tolyl)- , 1-methyl-6-(4–nitro phenyl)-, and 1-methyl-6-(4 –methoxy phenyl)- -2,5,7,8 tetrahydro-2-thio-pyrimido[4,5-d] pyrimidine 4-one, compounds **3,4** and **5** respectively showed enhanced inhibitory effect against Gram–positive bacteria and absence of the activity against *A. niger*. No changes were happened in the activity against *E. coli* and *C. trobicalis*. Substitution with ethyl group, **7**, showed a good activity against *A. niger*.

Table (1): Antimicrobial activity of the newly synthesized compounds

Compound	Ar. or R	Diameter of the inhibition zone (mm)				
		B. subtilis	***E. coli***	***C. tropicalis***	***A. niger***	**MIC against *B. subtilis* (µg/cm^3)**
TMAT		-	-	-	-	9
2	C_6H_5	10	10	0	8	-
3	p.CH_3.C_6H_4	**12**	10	0	0	20
4	p. NO_2.C_6H_4	**15**	**12**	0	0	12
5	p.OCH_3.C_6H_4	**12**	10	0	0	›20
6	naphthyl	10	10	0	0	-
7	C_2H_5	0	10	0	**12**	-
15	H	10	10	0	0	-
16	4.OH	0	10	0	**14**	-
17	2.OH	**12**	0	0	0	›20
18	4.Cl	**14**	0	0	0	20
19	4.Br	10	10	0	0	-
20	4.F	0	0	0	0	-

The thioxanthine derivatives also showed different activities according to the substituents in position 8 where substitution with 2 hydroxy phenyl and 4 chloro phenyl **17** and **18** respectively showed the best derivatives where showed a good inhibitory effect on Gram –positive bacteria only where all the other test organisms were resistant towards these compounds. At the same time 4 hydroxy phenyl derivative **16** showed a good inhibitory effect against *A. niger* and moderate activity against *E. coli.* As shown in Table (1) we can conclude that compound no **4** from the first group and **18** from the second group are promising compounds have good inhibitory activity against Gram-positive bacteria, where compound no **16** is the most active compound against *A. niger* followed by compound no **7**. *E. coli* showed moderate sensitivity towards the most of the synthesized compounds but *C. tropicalis* showed resistance towards all the tested compounds. It was clear that the type of the substituents affect on the activity of the derivatives where the nature of the substituents and the size of the atom can affect on the stability of the synthesized compound. MIC for the active compounds was determined and the results showed that the inhibitory effect of **4** extended until 12 µg/cm^3 demonstrated the high activity of this compound. This result is comparable with the reported data for other nucleotide analogues, 6-(3-ethyl-4-methylanilino) uracil (EMAU) and 6-(3,4-trimethylene) anilinouracil (TMAU), where they showed inhibitory effect against *B. subtilis* BD54 with *MIC* of 9 *µ*g / cm^3. Since Thiopurines are metabolized to deoxy-6-thioguanosine 5/-triphosphate via salvage pathway Lilla *et al.* (2003), examined the effects of a single thioG modification opposite cytosine on the structure and dynamics of duplex DNA in solution and showed the mechanism(s) by which this therapeutically relevant moiety elicits its biological activities. They found that due to the biomolecular compatibility, thioG is metabolised and ultimately incorporated into duplex DNA by DNA polymerase during replication. Other DNA processing enzymes (RNase H, Topo II, and DNA ligase) are not immune to the physical-chemical effects of thioG. These enzymes are exquisitely sensitive to the presence of thioG in DNA strands, and this may

due to the striking and localized effects of thioG on base pair stability and dynamics. So on the basis of structural and dynamic results they concluded that the thioG-modified duplex DNA has a lethal effect on the base pair stability. According to this described mechanism we can conclude that the different synthesized derivatives could be metabolized and incorporated into the duplex DNA resulted in lethal effects on the base pair stability.

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النشاط المضاد للميكروبات لمشتقات فيوسيد 6- اميتو 2- ثيوبراسيل جديده

سحره فتح الله محمد

قسم التكنولوجيا الحيويه الميكروبيه- المركز القومى للبحوث- الدقى- القاهره- مصر

تم فى هذا البحث اختبار مجموعتين من مشتقات الثيوبيورين كمضادات لكلا من البكتريا الموجبه والسالبة لجرام والفطريات كذالك الخمائر.

المجموعه الاولى هى مشتقات:- 1-methyl-6-substituted 2, 5, 7, 8 tetrahydro-2-thio-4-oxo-pyrimido [4, 5-d] pyrimidines (2-7).

اما المجموعه الثانيه فهى مشتقات:

8-substituted-3-methyl-7-hydroxy-2-thioxanthines (15-20).

حيث وجد أن هذه المركبات تتباين فى تأثيرها على كلا من البكتريا الموجبه والسالبه لجرام وان كانت أكثر تاثيرا على البكتريا من الفطريات بينما الخمائر لم تتأثر بأى من هذة المركبات تقريبا. ويمكن القول أن النشاط البيولوجى لهذه المركبات يعتمد على طبيعة الأستبدال على النوه الحلقيه.