The Antimicrobial Activity (Antibacterial, Antifungual) Of Some New 3-Substituted Isobenzofuran-1(3H)-One Derivatives

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Abstract

In this research a new series of 3-(substituted) isobenzohuran-1(3H)-one(B,D) and O-benzoyl benzamide derivatives (C,E) were studied successfully . The synthesis of these compounds has been described in the scheme 1. These products were screened for antifungal, antibacterial and enotoxic effect. It was found that all tested compounds have antifungal activity. Compound A1,A2,B2 and C3 were found to be active against Escherichia coli ,Bacillus subtilis and Staphylococcus aureus .Genotoxic effects using Ames test showed chart compound A1 and A2 have a weak base-pair substitution mutagenicity while a clear base- pair substitution mutagenic activity was shown by B4. Using TA100-strain of salmonella typhimurium compound D showed a framshift mutagenicity while a weak oxidative mutagenic action was revealed by compound E no change on the mutagenicty of the tested chemicals was observed after using the S9 metabolic action system.

(Hint: all of the organic compounds were prepared according to references [19].

الخلاصة

في هذا البحث تم بنجاح دراسة سلسلة جديدة من معوضات ايزوبنزوفيوران 1 (3H) – أون من نوع (B,D) و اورثو-بنزوايل بنزمايد من نوع (C , E). جميع المركبات تم فحصها كـ (antifungal, antibacterial and enotoxic effect) . وجد ان جميع المركبات اظهرت فعاليتها كمضادات فطرية . المركبات (A1 ,A2 ,B2 and C3) كان لها فعالية سمية ضد سلالات البكتريـة من نوع (Escherichia coli ,bacillus subtilis and staphylococcus aureus).من جهة اخرى اظهر تاثير الطفرات باستخدام (Ames Test) ان المركبات A1 , A2 لها نشاط ضعيف نسبيا تجاه تاثير mutagenicity بينما اظهرت المركب B4 تاثيره الواضح بهذا الاتجاه. عند استعمال الوسط TA100-strain of salmonella typhimurium اظهر انزياح مطفر للمركب D بينما كان تاثيره ضعيفا ومؤكسدا للمركب E. في حين لم يحدث اي تغير في عملية mutagenicity للمركبات المفاسة عند استخدام S9 metabolic action system .

(ملاحظة: جميع المركبات المدروسة حضرت طبقا للمصدر 19).

Introduction

The continuous appearance of resistant and mutiresistant pathogenic bacteria and fungi has promoted a continuous search for new antibacterial and /or antifungal drugs[1] Phthalide is an important structural moiety present in a variety of natural and synthetic products that possess significant biological activities[2]. For example , 3-n-butyl phthalide (NBP) exhibits antiasthmatic[3], anticonvulsant[4], activities and vasorelaxant [5],anti-inflammatory through protection of ischemic sites following ischemic brain injury [6] .

The concentration that gave the maximum number of his revertants were 74,260,215,55 and 27 μg/plate for compounds A1 ,A2 ,B4 ,D and E respectively .using the S9 metabolic activation system did not lead to any significant increase or decrease in the mutagenicity of the investigated compounds. Concerning the toxicity of the investigated compounds, it could be detected at a concentration ≥500 μg/plate in the S.typhomurium strains.

Experimental

Biological activities

Bacterial strains

The following strains were used in antimicrobial study; B.subtilis ATCC 6633, S. aureus ATCC 25923, E, coli ATCC 25922 and P.aeruginosa, and C.albicans. For mutagenicity test: S.typhimurium strains TA98, TA 100 and TA 102 were used. The last strains were kindly supplied by Prof.B.N. Ames (Department of Biochemistry, university of California, Berkely, U.S.A.).

Antimicrobial Activity

Preparation of the test chemicals: the test chemicals were dissolved in DMSO. Dextrose both was then added to get a starting concentration of 4mg/ml for each compound. The microorganisms were grown overnight in dextrose broth at 35°C and diluted to 10^{-3} just before being used. Plates were prepared by mixing each test chemical solution with the melted nutrient agar to get the desired final concentrations ranging form 450 down to 25μg/ml. The mixture were poured into Petri dishes and allowed to harden at room temperature. As positive controls, nalidixic acid as an antibacterial drug and miconazole as an antifungal drug were used. Each plate including positive and negative once was inoculated with a single streak using a 10μl calibrated loop. The plates were examined after 48hr incubation at 35 °C for the presence or absence of bacterial growth [9].

Mutagencity studies

Stock solution of the test chemicals were prepared by dissolving 10mg of the compound in dimethylsulfoxide .serial dilution ranging from 4 to 0.01mg/ml were made. Vogel-Bonner medium E(50X),histidine–biotin solution (0.5M),top agar, minimal glucose plates ,histidine –biotin and ampicillin plates were prepared as described by Maron and Ames [17]. The plates incorporation test as described by Maron and Ames was followed. The top agar was distributed into capped culture tubes which were held at 45°C in a water bath. To each tube, 0.1ml of a fresh overnight culture of the test strain was added, followed by the addition of 0.1 ml of the test compound. sodium azide ,nitrophenylene diamine and methylmethane sulfonate were used as apositive controls. The test components were mixed by vortexing the tube for about 3s at low speed and directly poured onto a minimal glucose agar plates. After 45min, the plates were inverted and placed in a dark 37°C incubator. The revertant colonies on the treated as well as on the negative control plates were counted .

The assay with S9mix was carried out as described by Maron and Ames [17]. The rat-liver S9fraction was prepared

as described by Ono et.al.[18]. Young male Sprague-Dawley rats, weighing approximately 200 mg,were supplied by yaromuk university / animal house unite (Jordan),and were used after induction with Phenobarbital and β-naphtofavone . the S9mix (50ml) contained 5ml of induced rat liver S9,of concentration was used . Triplicate plates were made for each dose, and each experiment was repeated at least twice in to separate days

Results and Discussion

Mahrath et al [7]. Reported that the reaction of 3-ethoxy phthalide with amino pyridine afforded N-(3-phthalidyl) amine type B, while Kenneth et al. [8] reported that the reaction of 3-halophthalides with cyclic secondary amines afforded N-(o-formylbenzoyl) amines, a product of type C. To our best knowledge the reaction of amines with 3-acetoxyphthalide A1 has not been reported .in our laboratory, we realized that the reactions of secondary amines with 3-acetoxyphthalide A1 afforded exclusively ; in opposite manner to that published by Kenneth et al.[8], N-(3-phthalidyl) amines (B1-4) except with diisopropylamine, the reaction afforded compound C1.

The two series of compounds investigated in the present study were synthesized as potential antimicrobial agents. The results of antimicrobial activity showed clearly that all tested compounds exhibited antifungal activity against yeast-like fungi, c.albicans (table 1) . This indicates that such types of compounds are good candidates to be used as fungicidal drugs.

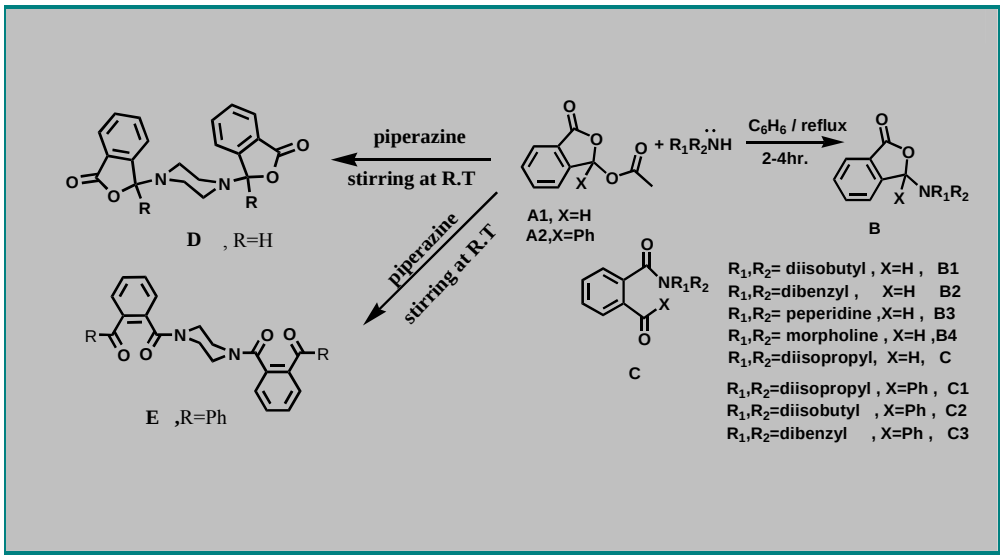
The antibacterial activity of the above compounds reveled a rather good activity against three out of four used strains. However ,such activity was shown by four compounds out of ten .this may be used as an indication to further improve such active compounds in order to both increase their activity against the fungi and the three strains of bacteria (table 1) and to broaden their spectrum against Gram positive and Gram negative bacteria. Development of the other related series of organic compounds may also be recommended.

In the genotoxicity studies we used Ames test. a great advantage of which is that it allows the investigator to differentiate between framshift,oxidative and base-pair substitution mutagens [10,11,17].in the present of investigation five compounds were found to be mutagenic although at relatively high concentrations. the results indicated also that the toxicity of such compound is not high in the salmonella strains used in this study. However, it is worthy mention that the mutagenicity of compounds A1, A2, B4 and E is weak.

Compound D, on the other hand, showed a clear frame shift mutagenic activity in TA98 strain. These findings may be attributed to the differences in chemical structures and /or chemical properties of the investigated compounds. Although four out of five compounds were weakly mutagenic, we recommend: first ,further investigation using different systems to confirm their genotoxic activity ; second ,to take these results into consideration if any practical use of these compounds is under discussion .

However, the rest of the compounds including those of potential promising activity as antimicrobial agents have not shown any genetic material-hazardous effect in the used system. Accordingly, further studies of these compounds concerning their mutagenicity using different system such as SOS chromo test [12, 13] or other test is highly recommended. Using mammalian

system for further investigation of the formerly described compounds such as toxicity testing, mouse micronucleus test, rat river UDS test, SCE in human lymphocytes and others is highly recommended [14-16].



Scheme 1

Table 1: Antifungal and antibacterial activities [conc.350 µg/ml]

Comp.no.	*C.albicans*	*B.subtilis*	*E. coli*	*P.aeruginosa*	*S.aureus*
A1	+	+	+	-	+
A2	+	+	+	-	+
B4	+	-	-	-	-
B3	+	-	-	-	-
B1	+	-	-	-	-
B2	+	+	+	-	(+/-)
D	+	-	-	-	-
C1	+	-	-	-	-
C2	+	-	-	-	-

Table 2: Mutagenic activity of studied compounds

Comp.no.	TA 98	TA 100	TA 102
A1	-	~ +	-
A2	-	~ +	-
B4	-	+	-
B3	-	+	-
B1	-	-	-
B2	-	-	-
D	+	-	-
C1	-	-	-
C2	-	-	~ +
C3	-	-	-

Table 3: Reversion of taster strains by compounds [A1 ,A2,B4 , D , E]

Comp. no.	Tester strains	Concentration µg/plate[(a)]	No.of revertents/plate[(b)]
A1	TA 100	260	94
A2	TA 100	215	107
B4	TA 100	55	217
D	TA 98	28	74
E	TA 102	75	180

[(a)] The concentration at which the highest mutagenic activity was obtained; [(b)] the average of two experiments after subtracting the spontaneous revertants.

Conclusion

In the present investigation, series of phthalidylamines and O-Benzoylbenzaimde derivatives were synthesized in a distinguish type of reactions. The compounds, which were synthesized as potential antimicrobial agents were found to exhibit both antibacterial and antifungal action. Some of the tested compounds showed a weak mutagenic activity. Using the S9 metabolic activation system failed to induce changes in the mutagenic effects of the investigated compounds**.**

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