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Synthesis, Characterization and Antimicrobial Evaluation of New Compounds Derived from 2-Mercaptobenzoxazole

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Abstract

The synthesis of a new series of 2-mercaptobenzoxazole hydrazide(2-benzo[d]oxazol-2-ylthio) acetohydrazide) derivatives (3a-c and 4a,b) from the use of various sulfonyl chlorides in dichloromethane and triethyl amine for the compounds **(3a-c)** and different aromatic aldehydes in the presence of glacial acetic acid for the compounds **(4a, b)**. The new compounds anti-microbial effectiveness has been evaluated in vitro against two of **G(+)Ve** and two of **G(-)Ve** bacteria and for fungal activity. In comparison to other compounds, compounds **(3a-c)** had the most anti-microbial action against *Candida albicans* and *pseudomonas aeruginosa*, respectively, among them compound 3a was more active and this could be due to the presence of (Cl) group which is more electronegative group, which is in charge of increasing the activity, while compound 4b was more active than compound 4a, which is inert, and this might be because the (OH) group has replaced the (CH_3) group, which is responsible for its activity through the production of hydrogen bonds or by making the molecule more polar. By using their "(melting point, Thin Layer Chromato-graphy , attenuated total reflectance-Fourier ransform infrared (ATR-FTIR), ^{1} HNMR)", the target compounds were identified.

Keywords: MBT 2-Mercaptobenzoxazole, Anti-microbial, Schiff base, Benzene Sulfonyl chlorides.

Introduction

Infectious illnesses are to blame for a significant number of deaths worldwide. [(1)] Multi-drug resistance diseases have been seen to climb quickly in recent years, leading to an increase in a number of public health issues. There are many diseases that are now difficult to treat with conventional antibiotic medication, as a result, there is a greater need to create innovative antimicrobial agent. [(2)] Heterocyclic compounds are those that have complicated toroidal components made up of atoms other than carbon. The more prevalent heterogeneous atoms are nitrogen, oxygen, and sulfur. [(3)] Heterocyclic compounds have considerably helped society and occupied a large amount of space in the form of several pharmaceuticals for the treatment of various diseases [(4)].

Combining heterocycles results in a fresh chance to produce brand-new multicyclic molecules using an increase in biological activity. These organic substances have sparked a lot of attention in the scientific community recently Since they have a wide range of biological effects [(5)]. Due to their usage as intermediates in the creation of novel biological materials, benzoxazole derivatives have grown significantly in relevance in recent years. [(6)] The vast range of pharmacological activity of benzoxazoles, including their antibacterial [(6)], antifungal [(7)], anticancer [(8),] anti-inflammatory [(9)] and antimycobacterial [(10)] effects, make them important in medicinal chemistry.

The essential scaffold 2-Mercaptobenzoxazole (MBT) is known to be involved in a number of biological processes, and its derivatives are produced all over the world for a range of uses. S-acethydrazide hydrazone and S-acyl derivatives were discovered to be helpful in the leather sector and to have antifungal and antibacterial properties. [(11)] Sulfonamides are a crucial pharmaceutical product that are used as medicinal agents against numerous diseases as a result of their essential biological function. [(12)] In the pharmaceutical sector, the sulfonamide moiety has clinical and therapeutic significance. The active pharmacophore ($-SO_2NH_2$) which is the sulfonamide moiety that exhibits a wide range of pharmacological actions, including antibacterial, antimalarial, insulin-releasing, anti-diabetic, anti-HIV, high ceiling diuretic, anti-thyroid, and anti-tumor effects. [(13)] Sulfonamide's primary function in

medicine is as an antibacterial agent since it contains the SO2NH- group. Due to the vast range of applications for sulfonamides, it is very desired to discover a powerful and effective sulfonamide medication with high biological activity. Therefore, the production of recently synthesized hybrid heterocyclic molecules is quite interesting. [14]

Hugo Schiff defined Schiff base compounds in 1864 as compounds having an azomethine group that originate from a reversible acid-catalyzed condensation reaction between primary amine and carbonyl compounds [15,16] with large number of biological effects, including antibacterial, antifungal, anti-inflammatory, and anticancer properties [17-22]. Shiff base of 2-Mercaptobenzoxazole with various aromatic aldehydes and benzene sulfonyl chlorides to produce the final compounds, this study intends to develop novel 2-mercaptobenzoxazole derivatives and investigate the bioactivity of a few of those derivatives.

Materials and Methods

The synthesis utilized only substances with the greatest analytical purity. The M.P(melting points) of the compounds that were ultimately synthesized were found (uncorrected) by using Thomas Hoover Apparatus. TLC and R*f* values for Intermediate and final synthesized were performed using ((aluminum-precoated silica gel 60 F254 sheets (Merck)), single circular spot resulted by the exposure to UV 254 nm light. A Shimadzu Fourier transform infrared (ATR-FTIR) spectrophotometer was used to calculate the infrared spectra in a KBr disc. There were taken ^{1}HNMR spectra on the Ultra shield spectrophotometer, made by BRUKER (500 MHz), DMSO-d6 was also employed as a solvent.

Synthesis of ethyl 2-(benzo[d]oxazol-2-ylthio) acetate (1) [23]

Mixture of 2- mercaptobenzoxazole (0.8 g, 0.005 mole) with ethyl bromoacetate (0.85 g, 0.005 mole) and (0.69 g, 0.005 mole) potassium carbonate in 60 ml of dry acetone, refluxed for 16 hours, monitoring by TLC. After that the mixture was filtered then solvent was evaporated under reduced pressure, brown oil was obtained. Yield: 46%, R*f*: 0.55, (ethyl acetate: Chloroform (5.5:2.5), FTIR (υcm^{-1}) :2985, 2873(C-H $_{aliphatic}$) str. for both asymmetrical and symmetrical, 1735(C=O of ester), 1600, 1504 (C=C of $_{aromatic}$), 1161 (C-O of ester).

Synthesis of 2-(benzo[d]oxazol-2-ylthio) acetohydrazide (2) [24, 25]

(1.07g ,0.004 mole) of compound 1 was refluxed with (2 ml, 0.04 mole) of 99% hydrazine hydrate in 35 ml absolute ethanol for 24 hour, the reaction was monitoring by TLC. And to get the final product the solvent was evaporated, ice was added and white solid powder was resulted after filtration, washing many times with cold D.W, drying and recrystallization with ethanol. Yield: 73%, R*f*: 0.6(Chloroform: ethyl acetate 7:3), M.P: 166-169 ºC, FTIR (υ cm^{-1}): 3302,3271(NH_2), 3167(N-H), 3059 (C-H $_{aromatic}$), 2962,2854(C-H $_{aliphatic}$),1635 (C=O) .^{1} HNMR δ_H (ppm, DMSO-d_6):8.94(1H,s,CO-NH),6.40-6.65(9H,m,Ar-H),6.38(2H,s,H-NH_2),4.48(2H,s, S- CH_2).

Synthesis of N'-(2-(benzo[d]oxazol-2-ylthio) acetyl)-subsitituted arylsulfonohydrazide (3a-c) [26]

(0.53 g, 0.002 mole) of compound 2 was dissolved in 35 ml of dichloromethane and different sulfonyl chloride compounds were added respectively p-chloro-benzene sulfonyl chloride (0.42 g, 0.002 mole), p – tolyl sulfonyl chloride (0.4 g, 0,002 mole), benzene sulfonyl chloride (0.6 g, 0.002 mol), in the presences of (0.002 mole, 2 ml) of triethylamine. The mixture was stirred overnight at room temp. The mixture was poured into a separatory funnel and washed with 60 mL of (D.W), after that the organic solvent was evaporated with reduced pressure to get the final compound (**3a-c**). Below is a list of Compounds 3a-c (appearance, Yield, M.P, R*f* value, and ^{1}HNMR and FTIR spectra):

N'-(2-(benzo[d]oxazol-2-ylthio) acetyl)-4-chlorobenzenesulfonohydrazide (3a)

Brown powder, Yield: 50%, M.p: 77-79 ºC, R.*f*: 0.59 (Ethyl acetate: n-Hexane 4:6), FTIR (υ cm^{-1}): 3410 (NH_2), 3097(C-H $_{aromatic}$),2954,2854 (C-H $_{aliphatic}$), 1620 (C=O), 1581(C=N), 1500,1458 (C=C $_{aromatic}$),1319,1153(SO_2) ,757 (C-Cl).^{1}HNMR (δ_H (ppm, DMSO-d_6):8.96(1H,s CO-NH),6.64-7.94(8H, m,Ar-H),6.47(1H,s SO_2-NH), 5.06(2H,s, CH_2).

N'-(2-(benzo[d]oxazol-2-ylthio) acetyl) benzenesulfonohydrazide (3b)

Dark brown powder, Yield 90%, M.p: 85-87 ºC, R.*f*: 0.53 (Ethyl acetate: n-Hexane 4:6), FTIR (υ cm^{-1}):3383 (NH_2), 3066 (C-H $_{aromatic}$) ,2927,2823(C-H $_{aliphatic}$) ,1627 (C=O), 1585(C=N $_{imine}$), 1500,1450(C=C $_{aromatic}$) ,1315,1192 (So_2). ^{1}HNMR δ_H (ppm, DMSO-d_6): 7.96 (1H, s CO-NH),6.71-7.80(9H, m, Ar-H),6.45(1H, s -SO_2-NH), 5.03 (2H, s, CH_2).

N'-(2-(benzo[d]oxazol-2-ylthio) acetyl)-4-methylbenzene sulfonohydrazide (3c)

Gray powder Yield: 56%, M.p: 89-91 ºC, R.*f*: 0.42 (Ethyl acetate: n-Hexane 4:6), FTIR (υ cm^{-1}): 3414 (NH_2), 3055 (C-H $_{aromatic}$), 2954, 2858(C-H $_{aliphatic}$), 1620 (C=O), 1597 (C=N) ,1500 -1458 (C=C $_{aromatic}$), 1365,1192 (SO_2). ^{1}HNMR (δ_H (ppm, DMSO-d_6): 8.97 (1H, s,CO-NH),6.37-7.82(9H,m, Ar-H),6.41(1H,s, SO_2-NH), 4.48 (2H,s, CH_2),2.50(2H,s, CH_3).

Synthesis of 2-(benzo[d]oxazol-2-ylthio)-N'-(subsitituted benzylidene) acetohydrazide 4a, 4b) (27,28)

(0.001 mole) of compound 2 in 40 ml of absolute ethanol was refluxed with different aromatic aldehyde (0.001 mole) with (5) drops of glacial acetic acid as catalytic agent for 12 hours, monitoring with TLC, the end product was obtained after solvent vaporization, filtration and recrystallization with ethanol. Below is a list of Compounds 4a, 4b Appearance, Yield, M.p, R_f value, and ^{1}HNMR and FTIR spectra:

2-(benzo[d]oxazol-2-ylthio)-N'-(4-hydroxy-3,5-dimethoxybenzylidene) acetohydrazide (4a)

Yellow powder, yield: 62%, m.p: 148-150 ºC, R.*f*: 0.7 (Chloroform: toluene: methanol 2:2:6). FTIR (υ cm^{-1}) : Broad band at 3514 due to (O-H) str. of phenol overlapping with (N-H) str. Of hydrazone), 3194 (NH_2), 3051(C-H $_{aromatic}$), 2966,2870(C-H aliphatic) ,1658 (C=O),1620 (C=N), 1557,1454 (C=C $_{aromatic}$). ^{1}HNMR (δ_H (ppm, DMSO-d_6): 10.50 (1H,s,C=O-NH_2), 9.05 (1H,s,O-H), 8.57 (1H,s, N=CH), 7.14-8.05 (6H,m, H-Ar),4.32(2H,s,S-CH_2),3.81(6H,s, O-CH_3).

2-(benzo[d]oxazol-2-ylthio)-N'-(2,4-dimethyl benzylidene) acetohydrazide (4b)

Orange powder, yield: 88%, m.p: 146-148 ºC, R.*f*: 0.6 (Chloroform: toluene: methanol 2:2:6). FTIR (υ cm^{-1}): 3178 (NH_2), 3070 (C-H $_{aromatic}$), 2947, 2866 (C-H $_{aliphatic}$), 1666 (C=O), 1604(C=N), (1508,1450(C=C $_{aromatic}$). ^{1}HNMR (δH (ppm, DMSO-d_6): 8.83(1H,s, C=O-NH_2),6.64 (1H,s, N=CH), 6.58-6.36(7H, m, H-Ar),3.84(2H,s, S-CH_2) , 1.91(6H,s, CH_3).

Antimicrobial Activity (29)

The final compounds were evaluated for their anti-microbial activity against four types of non-pathogenic bacteria two for gram positive strains (*Streptococcus pyogenes*, *Staphylococcus aureus*) and two for gram negative strains (*Escherichia coli*), (*pseudomonas aeruginosa*) and (*Candida albicans*) for fungi. This was done by a disk diffusion method; the culture medium was nutrient agar for bacteria and Sabouraud dextrose as growth medium for fungi. DMSO used as solvent to dissolve the tested compounds at concentration of 500 µg/mL, Ciprofloxacin, fluconazole used as references. The inhibitory zones were found after an incubation period of five days at 28°C for fungal and 24 hours at 35°C for bacteria.

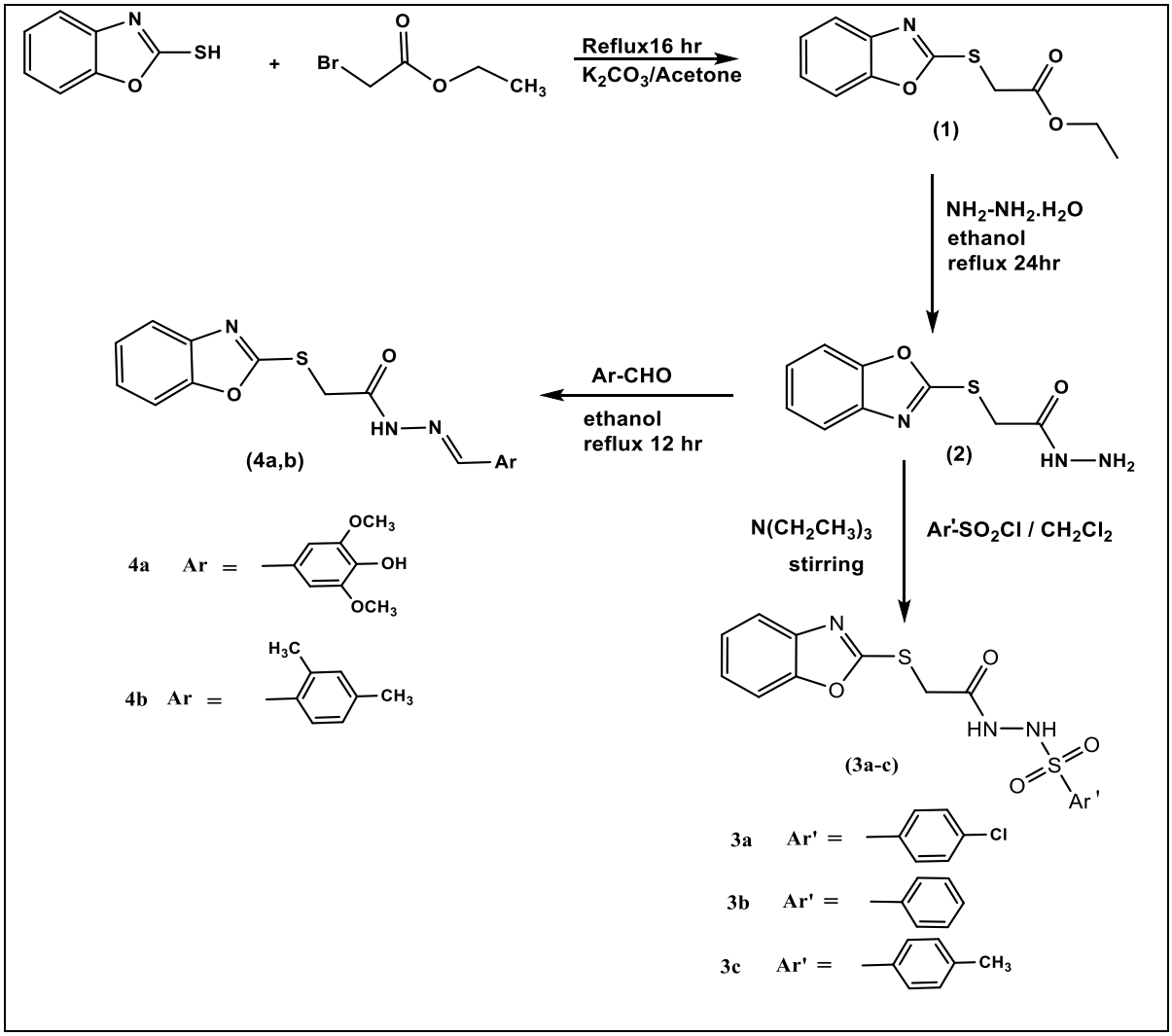
Results and Discussion

Chemistry

Scheme 1 summarizes the typical synthesis process for the 2-mercaptobenzoxazole derivatives (3a-c) and (4a, b). The first chemical, a 2-mercaptobenzoxazole ester (1), was created by combining 2-mercaptobenzoxazole with ethyl bromoacetate in dry acetone. The second compound was created by reacting compound 1 **w**ith a 99% hydrazine hydrate in ethanol then the reaction of compound **2** with various aryl Sulfonyl chloride compounds, including p-tolyl sulfonyl chloride, benzene sulfonyl chloride, and p-chlorobenzene-sulfonyl chloride, respectively, in dichloromethane in the presence of triethylamine as a base, to produce the target compounds (3a-c). The synthesis for the additional final compounds, N-acyl Hydrazones derivatives (4a,b), was carried out by reacting compound (2) with various aromatic aldehydes, 3,4-Dimethyl-benzyl aldehyde, 4-OH-3,5 dimethoxy benzaldehyde, in ethanol, and in the presence of glacial acetic acid. Infrared absorption spectra of these derivatives have verified their structures. For the final compounds (3a-c), disappearance for both asymmetrical and symmetrical absorption bands of amine group of the hydrazide (compound 2) at (3167,3271) with appearance of new peak at range (3383-3414) cm^{-1} which represent (N-H) stretching absorption peaks of amide, in addition to amide (C=O) stretching absorption peaks at range of (1620-1627) cm^{-1} and the presence of sulfone groups at range of (1356-1153) cm^{-1}, the disappearance of the ester group at (1735), represent the formation of these compounds, while for the compound (4a,b) the appearance of amide stretching at (1658-1666) with the imine stretching at (1604-1620) represent their formation. ^{1}HNMR of final synthesized compounds showed signals in (8.97- 7.96) ppm for NH protons, (7.82-6.37) ppm for aromatic protons.

Evaluation of anti-microbial activity

The newly developed compounds antimicrobial properties (3 a-c and 4a-b) were evaluated. Based on the outcome in table 1, when compared to Ciprofloxacin at a concentration of 500µg/mL. The compounds 3(a-c) demonstrated better efficacy against G (-) ve bacteria "*((Escherichia coli*, *pseudomonas aeruginosa*)) compared with G(+) ve bacteria ((*Streptococcus pyogenes*, S.*aureus*))" while the compounds 4(a,b) have the 4b compound more active than the 4a compound, which is inert. This might be because the (OH) group has replaced the (CH_3) group, which is responsible for raising the activity through the production of hydrogen bonds or by making the molecule more polar. All of them (aside from compound 4a which exhibited no activity) showed high activity for *Candida albicans*.



Scheme 1: synthesis of final target compounds (3 a-c, 4 a,b)

Table 1: Antimicrobial activity of the new compounds *in vitro* measured in mm.

Compounds	Zone of inhibition in mm					
	Conc.µg/mL	*S.aureus*	*S.pyrogen*	*E.coli*	*Pseudo.*	*C.albicans*
3a	500	10	11	12	21	23
3b	500	11	12	15	20	22
3c	500	-	18	11	15	20
4a	500	-	-	-	-	-
4b	500	15	13	13	14	21
Ciprofloxacin	500	16	17	20	24	15
Fluconazole	500	-	-	-	-	23
DMSO	**Solvent**					

No activity = (-), slightly active= (zone of inhibition between 5–10 mm), moderately active = (zone of inhibition between 10-15 mm), highly active = (zone of inhibition more than 15 mm)

Conclusion

By adhering to the previously specified processes, the synthesis of these suggested compounds was accomplished effectively. The findings of this study showed that the method used to create the intended derivatives was effective since the conformance of the compounds produced could be determined using information from physical and chemical investigations such melting point, FTIR and ^{1}HMNR. These substances have strong antimicrobial activity equivalent to commercially available products. Compounds 3(a-c) were more effective against G(-) Ve bacteria (mainly against *pseudomonas aeruginosa*) than G(+) Ve bacteria, among them compound 3a was more active and this could be due to the presence of (Cl) group which is more electronegative group, which is in charge of increasing the activity, while compound 4b was

more active than compound 4a, which is inert, and this might be because the (OH) group has replaced the (CH_3) group, which is responsible for its activity through the production of hydrogen bonds or by making the molecule more polar.

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Conflicts of interest

The authors affirm that there are no conflicts of interest and that no outside funding was used for the work

Author Contribution

Mohammed Kamil conceived the idea for our research. The experiment was completed by Zainab Dhia. Mohammed Abdulameer and Abdul-Hafeedh H. Abdul-Wahab assessed the antimicrobial activity. An explanation of the findings was written by Dr. Mohammad Kamil and Zainab Dhia.

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تحضير وتشخيص وتقييم مضادات الميكروبات للمركبات الجديدة المشتقة من ٢-مركبتوبنزوكسازول

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الخلاصة

تحضير سلسلة جديدة من مشتقات ٢-مركبتو بنزواوكسازول(٣ أ-ج)و(٤ أ-ب) عن طريق استخدام كلورايدات سلفونيل بنزين بوجود ثنائي كلوريد الميثين و ثلاثي اثيل امين للمركبات (٣أ-ج) و الديهايدات اروماتية مختلفة بوجود حامض الخليك الثلجي للمركبات (٤أ-ب), تم تقيم المركبات الجديده لفعاليتها المضاد للبكتيريا ضد اثنين من البكتريا الموجبة الغرام و واثنين من البكتريا السالبة الغرام وفعاليتها المضاده للفطريات خارج الجسم الحي.المركبات (٣أ-ب) كان لديها اقوى مضاد فطري وبكتيري بالتتابع مقارنة بالمركبات الأخرى. المركب (٣ج) كان لديه اقوى مضاد للبكتريا موجبة الغرام (البكتريا المكوره العقدية المقيحة) مقارنة بالمركبات الأخرى. تم تشخيص المركبات المستهدفة

الكلمات المفتاحية : ٢-مركبتو بنزواوكسازول, مضاد للالتهاب البكتيري, قواعد الشف, كلورايدات سلفونيل البنزين