

Synthesis and Evaluation of Antimicrobial activity of several new Maleimides to Benzothiazole moiety

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Abstract:

In this work, a series of new maleimides linked to substituted benzothiazole moiety were synthesized. Synthesis of these new cyclic imides were performed via three steps, the first one involved preparation of a series of 2-aminobenzothiazole substituted with different substituents via reaction of different primary aromatic amines with ammonium thiocyanate and bromine in glacial acetic acid. The prepared 2- amino benzothiazoles were introduced in the second step in reaction with maleic anhydride producing a series of N-(substituted benzothiazole-2-yl) maleamic acids. The resulted maleamic acids were dehydrated in the third step via treatment with acetic anhydride and anhydrous sodium acetate to afford a series of the desirable N-(substituted benzothiazole -2-yl) maleimides. The synthesized maleimides were screened for their antibacterial activity against two types of bacteria including (staphylococcus aureus) Gram positive and (Klebsiella pneumoniae) Gram negative bacteria respectively. Antifungal activity of the prepared imides also were tested against (Candida albicans) fungi. The new compounds were found to exhibit good antibacterial and antifungal activities.

Key words: Synthesis ,Antimicrobial activity , Maleimides Benzothiazole

Introduction:

Benzothiazoles are heterocyclic compounds with multiple applications and although they have been known from long ago to be biologically active[1]

Their varied biological features are still of great scientific interest nowadays. They show for example very intensive antitumor activity[2][3] especially the phenyl substituted benzothiazole while other benzothiazoles exert antiviral activity[4] . Benzothiazoles are also found to have antimicrobially([5][6] anticancer and anti-inflammatory[7] activities.

On the other hand synthetic cyclic imides such as succinimides , maleimides phthalimides and related

compounds possess structural features which confer potential biological activity and pharmaceutical use. All of the various classes of cyclic imides have received attention due to their antibacterial, antifungal , analgesic and antitumor activities [8-10]

All these biological activities prompted as to synthesis several new cyclic imides containing benzothiazole moiety with expected biological activity.

Materials and Methods :

Melting point were determined by Gallon kamp capillary melting point apparatus and were uncorrected .FTIR spectra were recorded using KBr disc on Shimadzu FTIR-8400 Fourier transforms infrared spectrophotometer.

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U.V spectra were recorded using shimadzu U.V -visible recording spectrophotometer U.V 160. ¹H-NMR spectra were recorded on near magnetic resonance Bruker , Ultrashield 300 MHz, using deuterated DMSO as solvent and TMS as internal standard. Incubator Hetashi model was used for incubation samples in biological study. All chemicals employed in this work were from BDH , Fluka and Merk.

1-Preparation of substituted -2-amino benzothiazoles [1-10]

The titled compounds were prepared according to literatures ⁽¹¹⁾ with some modification .

A mixture of (0.03 mole) of P-substituted aromatic primary amine, (0.1 mole) of ammonium thiocyanate and (150 ml) of glycol acetic acid was placed in a suitable round bottomed flask, then (1.2 ml) of bromine dissolved in (75 ml) of glycol acetic acid was added drop wise during 1.5 hrs with stirring and cooling.

When addition was completed the mixture was stirred for additional 15 min. then was made alkaline by addition NaOH solution and the optional precipitate was filtered ,dried then purified by recrystallization from suitable solvent. Physical properties of the prepared benzothiazoles [1-10] are shown in table (1):

2- Preparation of N-(substituted benzothiazole-2-yl) maleamic acids[11-20]

(0.01 mole) of maleic anhydride was dissolved in (80 ml)of dry acetone in a suitable round bottom flask fitted with dropping funnel which was supplied with (0.01 mole) of substituted-2-amino benzothiazole dissolved in (30 ml) of dry acetone.

The solution in dropping funnel was added drop wise to the mixture with stirring and cooling⁽¹²⁾.

When addition was completed stirring was continued for one hour then the precipitated amic acid was filtered of

,washed with diethyl ether and dried .Amic acid was purified by recrystallization from a suitable solvent but when this method was not successful purification was performed by dissolving the amic acid in a dilute solution of NaHCO₃ followed by precipitation by dilute HCL.Physical properties of the prepared maleamic acids [11-20] are shown in table (2).

3-Preparation of N-(substituted benzothiazole-2-yl) malimides[21-30]

The titled imides were synthesized according to literatures⁽¹³⁾with minor modification:

A mixture of (0.01 mole) of N-(substituted benzothiazole-2-yl) maleamic acid in (20 ml) of acetic anhydride and (5%) by amic acid weight of anhydrous sodium acetate was refluxed with stirring for two hours.The resulted homogenous solution was cooled to room temperature then poured into excess cold water with vigorous stirring .The obtained precipitate was filtered , washed with water , dried and finally purified by recrystallization from suitable solvent . Physical properties of the prepared maleimides [21-30] are shown in table (3).

4- Biological study:

The cup plate method using nutrient agar media was employed in studying the antibacterial and antifungal activities of compounds [21-30] against staphylococcus aureus , Klebsiella pneumoniae and candida albicans fungi.

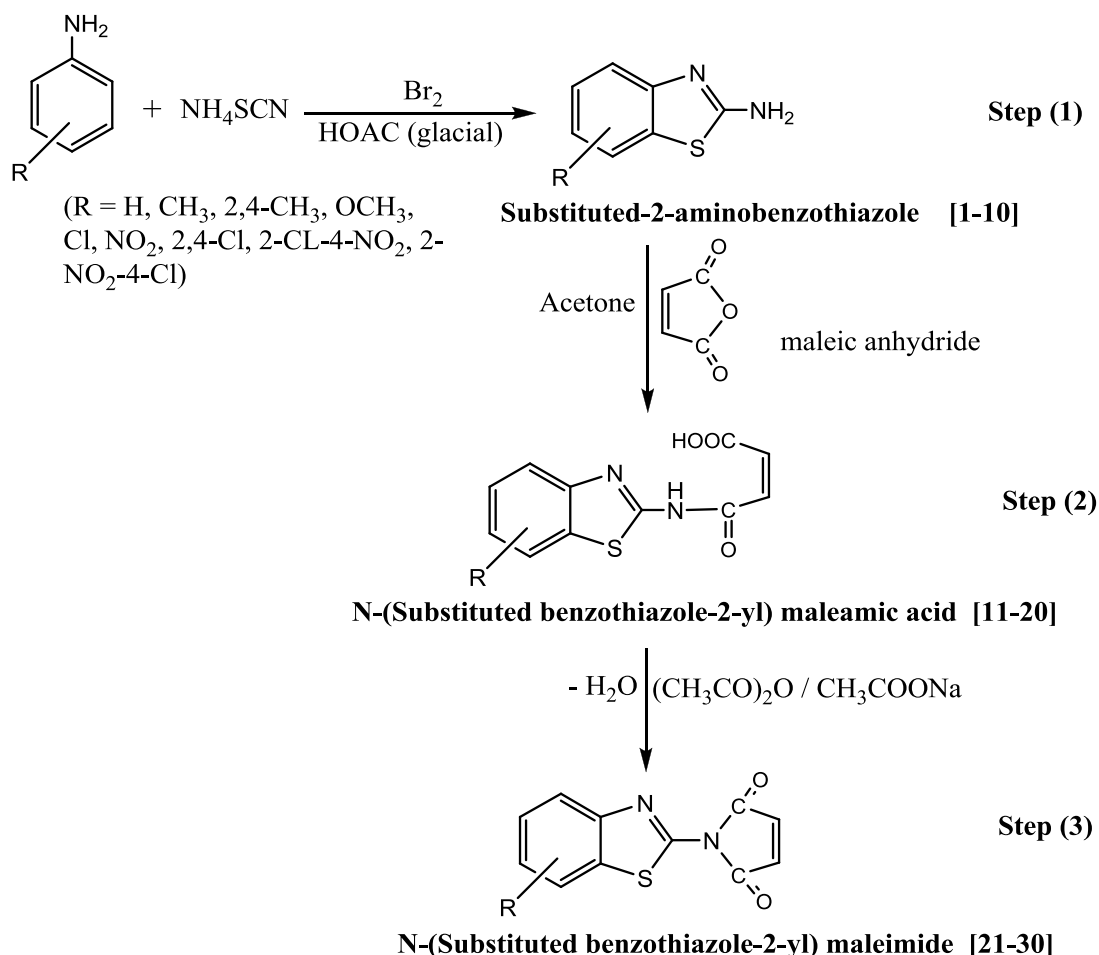
Using a sterilized cork borer cups were scooped out of agar media contained in a petridish which was previously inoculated with the microorganisms. The test compound solution (0.1 ml) was added in the cups and the petridishes were subsequently incubated at 37 c° for 48 hrs.

Zones of inhibition produced by each compound was measured in (mm) and the results are listed in table (7).

Results and Discussion:

Since both maleimides and benzothiazoles are known biologically active compounds the target of this work was directed toward synthesis of new compounds containing these two active moieties with expected biological activity. The strategy involved preparation a series of 2-aminobenzothiazoles which

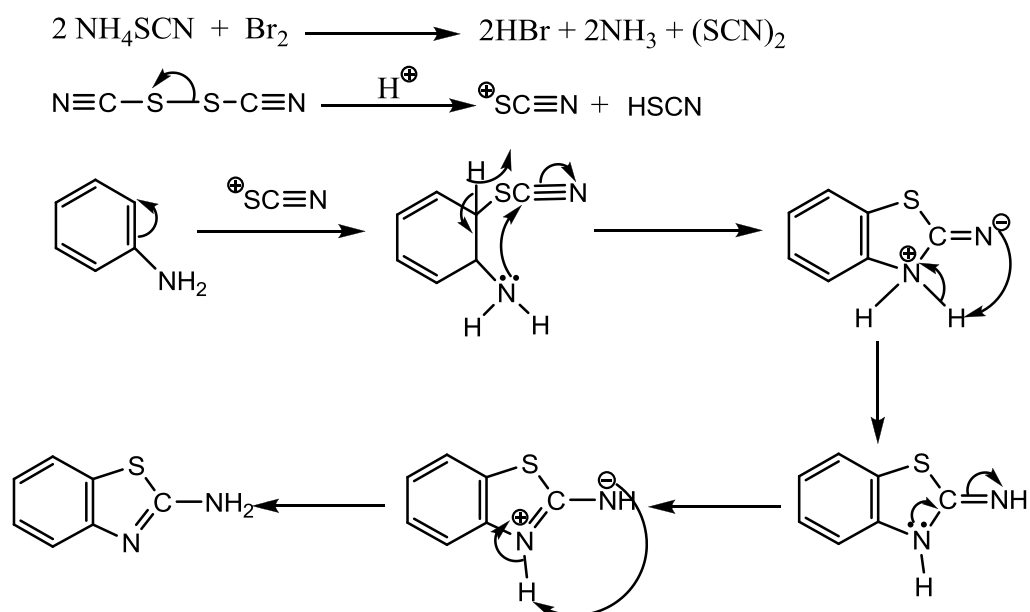
subsequently were introduced in reaction with maleic anhydride in the second step producing a series of the corresponding maleamic acids. Dehydration of the prepared maleamic acids in the third step gave the desirable maleimides. This linear pathway strategy can be summarized in scheme (1).

**Scheme (1)**

The first step in this work involved synthesis of ten 2-aminobenzothiazoles substituted with different substituents by applying thiocyanogen method which involved treatment of primary aromatic amines with ammonium

thiocyanate and bromine in glacial acetic acid.

This method involved introducing of thiocyanate group in primary amine followed by ring closure affording the 2-aminobenzothiazoles⁽¹¹⁾.



Scheme (2)

The resulted benzothiazoles are colored solids having sharp melting points and were afforded in high yields. Physical properties of the prepared benzothiazoles [1-10] are shown in table(1).

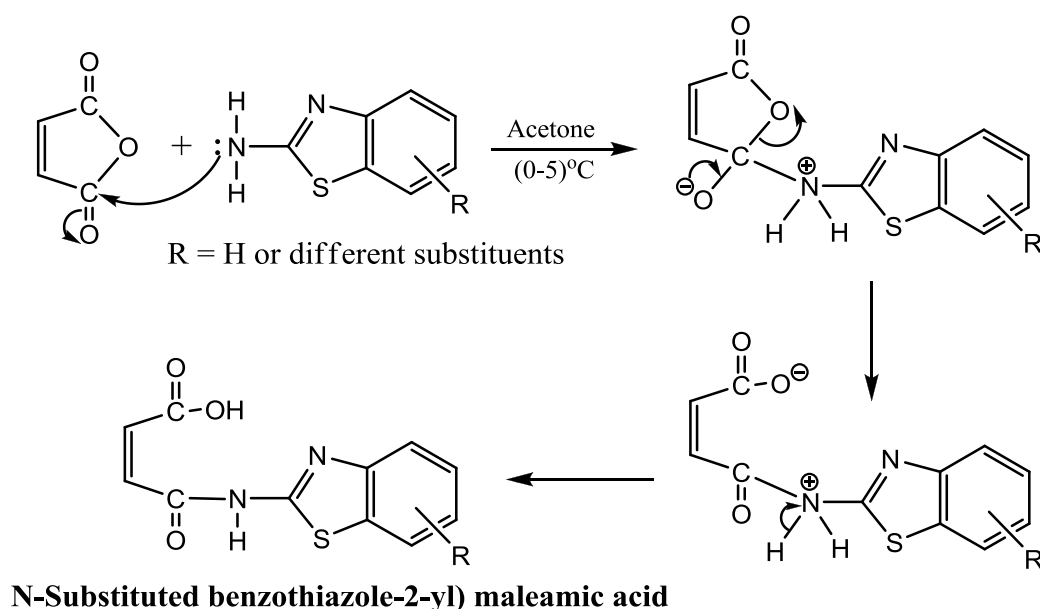
FTIR spectra of compounds[1-10] showed clear absorption bands at $(3263-3487) \text{ cm}^{-1}$, $(1442-1650) \text{ cm}^{-1}$ and $(709-617) \text{ cm}^{-1}$ due to $\nu(\text{NH}_2)$, $\nu(\text{C}=\text{N})$ thiazole, $\nu(\text{C}=\text{C})$ aromatic and $\nu(\text{C}-\text{S})$ thiazole respectively.

U.V spectra of compounds [1-10] showed absorptions at wave lengths $(240-378)$ due to $(\pi-\pi^*)$ and $(n-\pi^*)$ electronic transitions. HNMR clear singlet signal at $\delta = 2.3$ ppm belong to methyl group protons, signal at $\delta = 3.37$

ppm belong to (NH_2) protons and multiplet signals at $(\delta = 6.99-7.42)$ ppm belong to aromatic protons. FTIR and U.V spectra data of compound [1-10] are shown in table (4).

The second step in this work involved synthesis of ten N-(substituted benzothiazole-2-yl)maleamic acids [11-20]. Synthesis of these acids were performed via reaction of equimolar amounts of maleic anhydride and 2-aminobenzothiazoles (as primary amines).

Mechanism of this reaction involved nucleophilic attack of amino group of primary amine on carbon atom of one carbonyl group in maleic anhydride as shown in scheme(3).



Scheme (3)

FTIR spectra of the prepared amic acids [11-20] showed absorption bands at $(3178-3502) \text{ cm}^{-1}$ which belong to ν (O-H)carboxylic and ν (N-H) amide. Other bands appeared at $(1635-1704) \text{ cm}^{-1}$, $(1600-1643) \text{ cm}^{-1}$ and $(645-695) \text{ cm}^{-1}$ due to ν (C=O)carboxylic, ν (C=O)amide and ν (C-S) in thiazole ring respectively.⁽¹⁴⁾

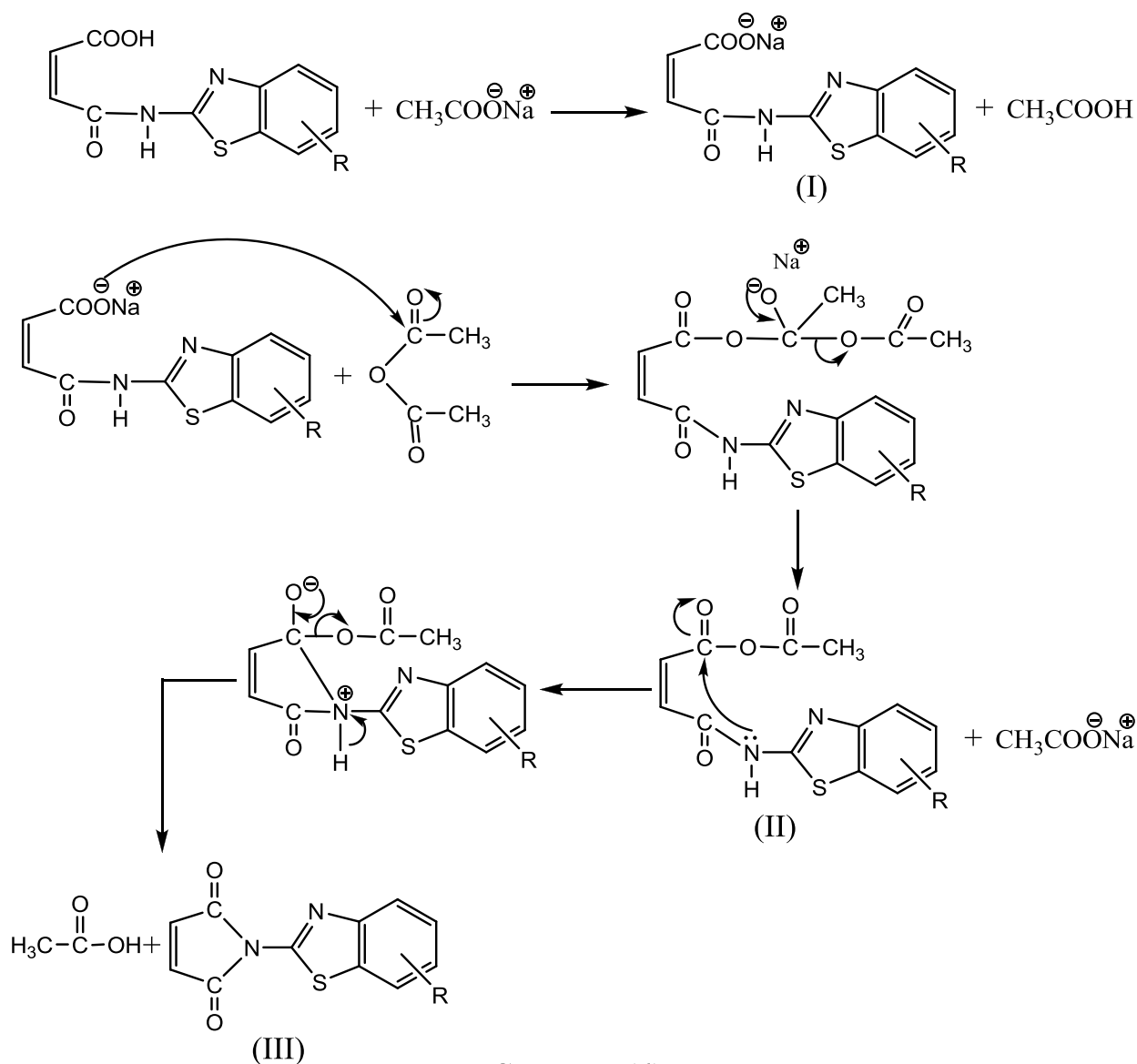
U.V spectra of compounds [11-20] showed absorptions at wave lengths $(244-299) \text{ nm}$ and $(301-342) \text{ nm}$ which attributed to $(\pi-\pi^*)$ and $(n-\pi^*)$ transitions in benzothiazole conjugated system and attached maleamic acid moiety.

On the other hand HNMR spectrum of amic acid [13] N-(4,6-dimethyl benzothiazole-2-yl) maleamic acid showed two singlet signals at $(\delta=2.3$ and $2.4) \text{ ppm}$ belong to protons of two methyl groups, two signals at $(\delta=6.2$

and $6.5) \text{ ppm}$ belong to two vinylic protons, signals at $(\delta=7-7.45) \text{ ppm}$ belong to aromatic protons and signals at $(\delta=7.65$ and $7.75) \text{ ppm}$ belong to (OH carboxylic) and (NH amide) protons respectively.⁽¹⁵⁾

FTIR and UV spectra data of compounds [11-20] are listed in table (5).

The third step in this work involved dehydration of the prepared maleamic acids. Dehydration was performed by using acetic anhydride and anhydrous sodium acetate as dehydration agent⁽¹³⁾. Mechanism of this reaction involved abstraction of proton from amic acid by catalyst anhydrous sodium acetate producing (maleamate ion I) which in turn attacked acetic anhydride producing (maleamic anhydride II) followed by ring closure as described in scheme (4).



Scheme (4)

Physical properties of maleimides [21-30] are shown in table (3).

FTIR spectra of prepared maleimides [21-30] showed disappearance of ν (O-H) and ν (N-H) absorption bands indicating successful dehydration reaction.

The spectra showed also clear absorption bands at $(1689-1728)\text{cm}^{-1}$, $(1504-1589)\text{cm}^{-1}$, $(1550-1650)\text{cm}^{-1}$, $(1334-1380)\text{cm}^{-1}$ and $(640-702)\text{cm}^{-1}$ which belong to ν (C=O) imide, ν (C=N) thiazole, ν (C=C) aromatic, ν (C-N) imide and ν (C-S) thiazole respectively.

U.V spectra of compounds [21-30] showed absorptions at wave lengths

$(230-281)\text{nm}$ and $(301-390)\text{nm}$ due to $(\pi-\pi^*)$ and $(n-\pi^*)$ transitions in benzothiazole moiety which was in conjugation with maleimide moiety. Details of FTIR and U.V spectra data of maleimides [21-30] are listed in table (6).

¹H NMR spectrum of compound [23] showed signals at $(\delta=2.1$ and $2.2)$ ppm belong to protons of two methyl groups, signals at $(\delta=6.5$ and $6.6)$ ppm belong to vinylic protons and signals at $(\delta=7.2-7.7)$ ppm belong to aromatic protons.

Biological activity:

The prepared imides were screened for their antimicrobial activity⁽¹⁶⁾ against

two types of bacteria (staphylococcus aureus , Klebsiella pneumoniae) and against Candida albicans fungi and

they showed different biological activities against these organisms as shown in table (7).

Table (7) Inhibition zones in(mm) of maleimides [21-30]against the tested organisms

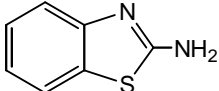
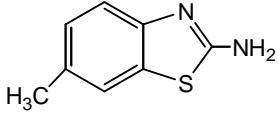
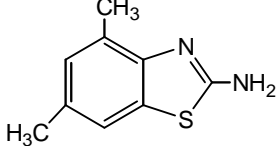
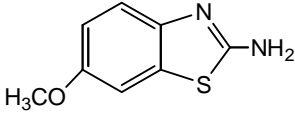
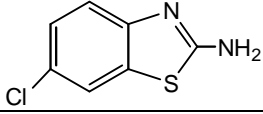
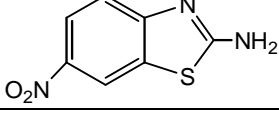
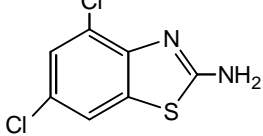
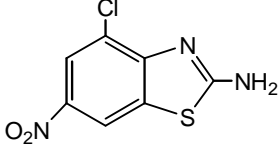
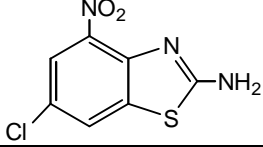
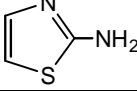
Comp. No.	Gram positive	Gram negative	<i>Candida albicans</i>
	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	
21	3	3	N
22	10	N	15
23	9	N	8
24	6	N	6
25	5	N	9
26	7	15	12
27	12	5	4
28	11	11	N
29	9	15	N
30	10	4	15

N = no inhibition.

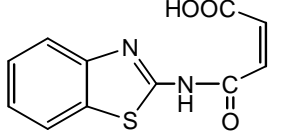
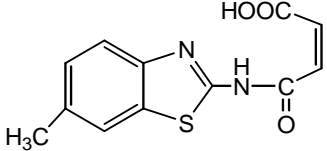
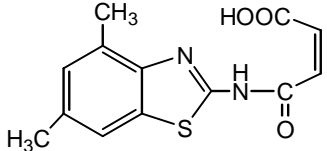
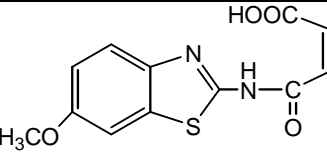
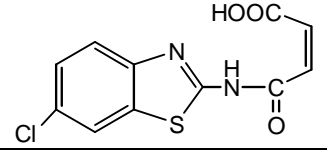
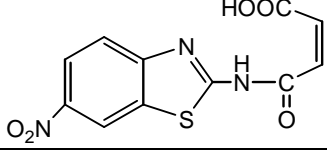
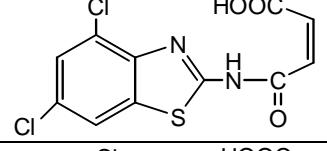
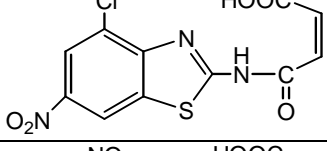
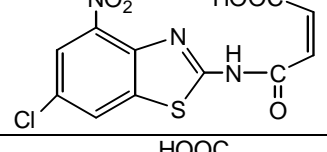
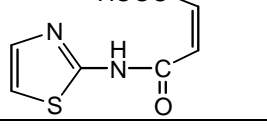
The results showed that antibacterial and antifungal activity of the prepared maleimides depend on nature of substituents in their molecules thus compounds [26] , [28] and [29] which are substituted with nitro groups showed high antibacterial activity against *Klebsiella pneumoniae* while other maleimides showed no activity against this bacteria.

Compounds [27] and [28] showed high activity against *staphylococcus aureus* , compounds [22] , [23] , [24] , [26] , [29] , and [30] showed moderate activity and compounds [21] and [25] showed weak activity against this bacteria. Finally compounds [22] , [26] and [30] showed high antifungal activity against *Candida albicans* fungi , compounds [23 -25] showed moderate activity , compound [27] showed weak activity and compounds [21] , [28] and [29] showed no activity against this fungi.

Table(1) Physical properties of the prepared substituted-2-aminobenzothiazoles

Comp. No.	Compound structure	Color	Melting point °C	Yield %	Recrystallization solvents
1		White	129-130	90	Benzene
2		White	136-137	95	Benzene
3		Faint yellow	131-133	90	Benzene
4		Violet	157-159	92	Benzene
5		Faint yellow	186-187	85	Benzene
6		Orange	122-124	87	Benzene
7		Faint yellow	240-241	71	Benzene
8		Deep yellow	92-94	89	Ethyl acetate
9		Orange	110-111	94	Ethyl acetate
10		Yellow	91-93	-	Ethanol

Table(2) Physical properties of the prepared amic acids

Comp. No.	Compound structure	Color	Melting point °C	Yield %	Recrystallization solvents
11		Off White	188-189	77	Methanol
12		White	179-180	65	Methanol
13		Faint yellow	140 dec.	80	Ethanol
14		Deep yellow	167-168	71	Ethanol
15		Faint yellow	192-194	84	Methanol
16		Deep yellow	170-171	58	Methanol
17		Faint yellow	200 dec.	66	Methanol
18		Deep yellow	120 dec.	57	Methanol
19		Deep yellow	100-102	55	Methanol
20		Deep yellow	132-134	53	Methanol

Table(3) Physical properties of the prepared maleimides

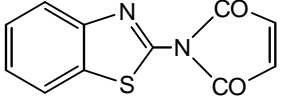
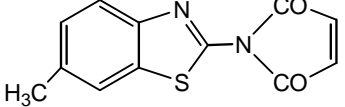
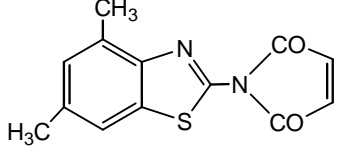
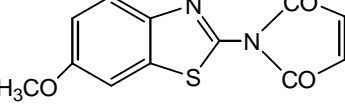
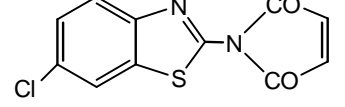
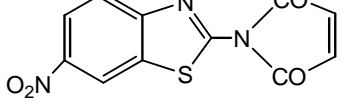
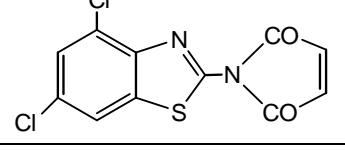
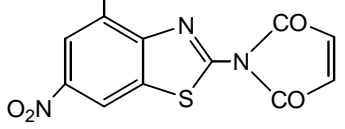
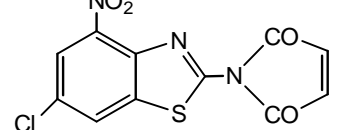
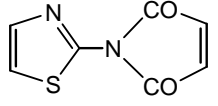
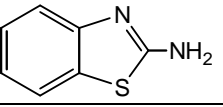
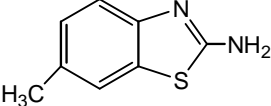
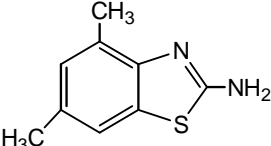
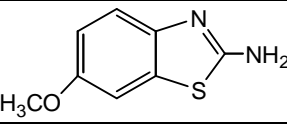
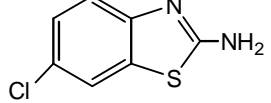
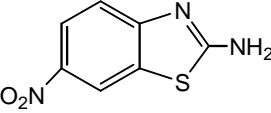
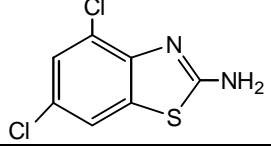
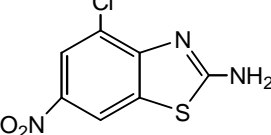
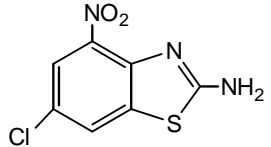
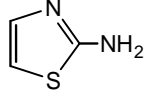
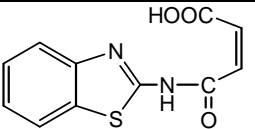
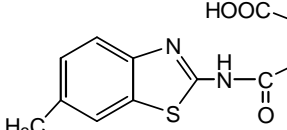
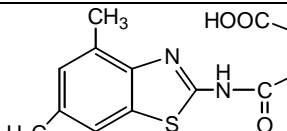
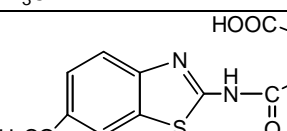
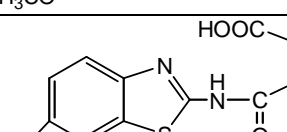
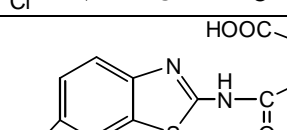
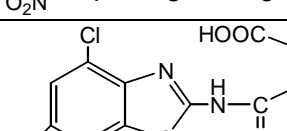
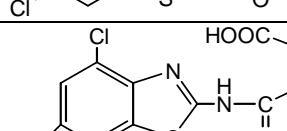
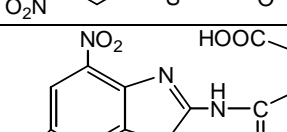
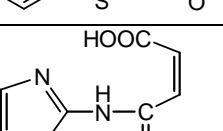
Comp. No.	Compound structure	Color	Melting point °C	Yield %	Recrystallization solvents
21		Off White	170-171	75	Cyclohexane
22		Faint yellow	144-145	69	Cyclohexane
23		Black	236-237	65	Cyclohexane
24		Faint yellow	150 dec.	81	Cyclohexane
25		Deep green	199 dec.	85	Cyclohexane
26		Green	155 dec.	90	Cyclohexane
27		Deep green	180 dec.	55	Cyclohexane
28		Dark brown	160 dec.	57	Cyclohexane
29		Dark brown	68-70	60	Cyclohexane
30		Faint yellow	140-141	62	Cyclohexane

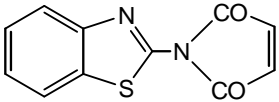
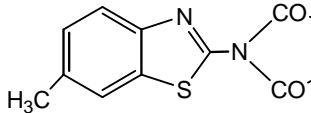
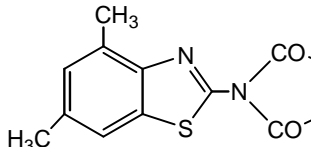
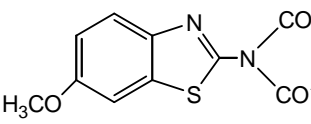
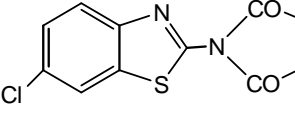
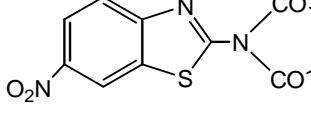
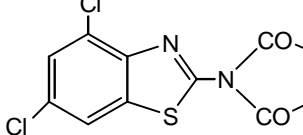
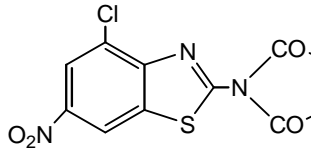
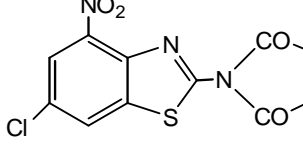
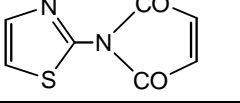
Table (4): Spectral data of the prepared N-Substituted-2-aminobenzothiazole

Comp. No.	Compound structure	FTIR Absorption Data (cm ⁻¹)					U.V Data	
		$\nu(\text{N-H})$ amine	$\nu(\text{C=C})$ and $\nu(\text{C=N})$	$\nu(\text{C-N})$	$\nu(\text{C=S})$	$\nu(\text{C-H})$ aromatic	Other bands	λ_{max} (nm)
1		3394 3271	1643 1527	1311	632	3055	—	252
2		3394 3263	1627 1542	1288	640 700	3124	—	252
3		3463 3355	1643 1604	1296	678	3070	—	251
4		3386 3294	1643 1550	1334	709 617	3101	1203 C-O-C	252,266
5		3456 3271	1635 1535	1280	702 648	3093	1103 C-Cl	253,287
6		3456 3348	1650 1540	1303	700	3062	1340 NO ₂	344,378
7		3487 3371	1636 1575	1319	678	3070	1095 C-Cl	259
8		3487 3371	1627 1581	1311 1265	640	3201	1370 NO ₂ 1126 C-Cl	345,375
9		3471 3355	1643 1566	1340	648	3178	1340 NO ₂ 1045 C-Cl	240,415
10		3317	1627	1342	617	—	—	244

Table(5) Spectral data of the prepared amic acids [11-20]

Comp. No.	Compound structure	FTIR Absorption Data (cm ⁻¹)							U.V Data λ_{\max} (nm)
		$\nu(\text{O-H})$ carboxylic	$\nu(\text{N-H})$ Amide	$\nu(\text{C=O})$ carboxylic	$\nu(\text{C=O})$ Amide	$\nu(\text{C=N})$	$\nu(\text{C-S})$	Other bands	
11		3178	3178	1666	1620	1535	640 695	—	294
12		3394	3471	1658	1604	1535	671	—	325
13		3417	3494	1704	1630	1550	655	—	272
14		3209	3440	1635	1604	1542	671	1226 C-O-C	299, 277, 252
15		3201	3201	1658	1596	1542	648	1103 C-Cl	324
16		3193	3355	1670	1635	1519	645	1334 NO ₂	301
17		3425	3425	1697	1643	1558	648	1050 C-Cl	342, 267
18		3379	3502	1697	1625	1510	648	1080 C-Cl	270
19		3355	3471	1704	1635	1504	655	1118 C-Cl	282
20		3093	3417	1650	1620	1550	648	—	244

Table(6) Spectral data of the prepared maleimides [21-30]

Comp. No.	Compound structure	FTIR Absorption Data (cm ⁻¹)						U.V Data λ_{\max} (nm)
		ν C=O Imide	ν C=N thiazole	ν C=C vinylic	ν C-N	ν C-S thiazole	Other bands	
21		1697	1550	1600	1365	678	—	249
22		1728	1545	1604	1350	702	—	262
23		1720	1558	1650	1373	686	—	266, 252, 243
24		1689	1558	1604	1434	671	1260 C-O-C	304
25		1704	1550	1596	1365	666	1103 C-Cl	249
26		1704	1520	1560	1342	665	1342 NO ₂	320
27		1720	1589	1650	1380	675	1075 C-Cl	276, 257, 230
28		1712	1512	1550	1340	640	1049 C-Cl	301, 281, 239
29		1712	1573	1600	1334	655	1360 NO ₂	344, 390
30		1728	1504	1596	1342	666	—	259

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تحضير وتقدير الفعالية المضادة للجراثيم لعدد من المالي ايميدات الجديدة المرتبطة بمكونة البنزو ثايازول

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الخلاصة:

تضمن البحث تحضير سلسلة من المالي ايميدات الجديدة المرتبطة بمكونة البنزو ثايازول. تم تحضير هذه الايميدات الحلقية الجديدة من خلال انجاز ثلاث خطوات تضمنت الخطوة الاولى تحضير سلسلة من مركبات 2-أمينوبنزو ثايازول المعوضة بمجاميع مختلفة من خلال تفاعل الامينات الاروماتية الاولى مع ثايوسيانات الامونيوم والبروم في حامض الخليك الثلجي. أما الخطوة الثانية فقد تم ادخال مركبات 2-أمينوبنزو ثايازول المحضرة في تفاعل مع انهيدريد المالك مما اسفر عن تكوين سلسلة من حوامض ن-(معوض بنزو ثايازول -2-يل) مالي اميك والتي بدورها سحب الماء منها في الخطوة الثالثة من خلال معاملتها مع انهيدريد الخليك وخلات الصوديوم اللامائية لانتاج سلسلة من الايميدات الجديدة المرغوبة ن-(معوض بنزو ثايازول -2-يل) مالي ايميد.

درست الفعالية البايولوجية للايميدات الحلقية ضد نوعين من البكتريا هما (ستافيلوكوكاس اورييس) الموجبة لصبغة غرام و (كلبيسيلا نيومونيا) السالبة لصبغة غرام.

كما درست فعالية الايميدات المحضرة ضد احد انواع الفطريات هو (كانديدا البيكانس) وقد اوضحت نتائج الدراسة بأن معظم الايميدات المحضرة ذات فعالية جيدة ضد انواع البكتريا والفطريات قيد الدراسة.