Synthesis and Evaluation of Biological Activity of Several New citraconimides Substituted with Benzothiazoles

BY

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Abstract

The target of the present work is to synthesize a series of new Citraconimides containing benzothiazole rings. Syntheses of these new cyclic imides were performed via two steps:

The first one involved preparation a series of N-(substituted benzothiazole-2-yl) Citraconamic acids via reaction of citraconic anhydride with 2-aminobenzothiazole substituted with different substituent's. The resulted citraconamic acids were dehydrated in the second step via treatment with acetic anhydride and anhydrous sodium acetate or by fusion method to afford a series of desirable N-(substituted benzothiazole-2-yl) Citraconimides. The synthesized compounds were screened for their antibacterial activity against four microorganisms' including [Staphylococcus aurous and Streptococcus pyogenes] (Gram positive) bacteria and [E.coli and Pseudomonas aeruginosa], (Gram-negative) bacteria respectively. The new compounds were found to exhibit good to moderate antibacterial activity .also anti fungal activity of the prepared compounds were tested against [Candida albicans] fungi and some of the tested compounds were found to exhibit good antifungal activity.

Introduction

Cyclic imides are an important functionality which have been found to maintain significant biological activity (1, 2) and represent an important moiety in creation of novel medical materials, thus some of them are used to therapeutic of arthritis, epilepsy and petitmal(3) while others used to treatment of tubercular bacillus , nematode and for inhibition the growth of poliovirus (4).

On the other hand 2- AminoBenzothiazole derivatives are an important class of hetero cyclic compounds which have long been recognized as therapeutic active skeletons and are useful for making antitumor agents, antibacterial, antifungal, and anti malarial and other biological activates (5-8). According to these facts the present work was directed toward synthesis of new cyclic imides (citraconimides) containing benzothiazole moiety in their structures followed by their antimicrobial screenings.

Experimental

Melting points were determined by Gallen Kamp capillary melting point apparatus and were uncorrected. FTIR spectra were recorded using KBr discs on Shimadzu FTIR-8400 Fourier transforms infrared spectrophotometer. U.V spectra were recorded using Shimadzu u.v –visible recording spectrophotometer u.v 160. H-NMR and C13-NMR spectra were recorded on near magnetic resonance Bruker, Ultrasheild 300 MHz,using deuterated DMSO, chloroform and methanol as solvent and TMS as internal standard. Incubater Hetashi model was used for incubation samples in biological study. All chemicals employed in this work were form BDH,Fluka and Merk.

1- Preparation of N-(substituted benzothiazol-2-yl) Citracon Amic acids [1-12]

(0.01 mol) of citraconic anhydride was dissolved in (30 mL) of dry acetone in a suitable round bottomed flask fitted with dropping funnel which was supplied with (0.01 mol) 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 (9). When addition was complete stirring was continued for one hour then the precipitated amic acid was filtered off, 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 dilute solution of sodium bicarbonate followed by precipitation by dilute hydrochloric acid. Physical properties of amic acids [1-12] are shown in Table (1).

2- Preparation of N-(Substituted benzothiazol-2-yl) Citraconimides [13-24]

Dehydration of the prepared Citraconamic acids to the corresponding citraconimides was performed by two methods:

2-1- Dehydration by Using Fusion Method

(0.01 mol) of N-(substituted benzothiazol-2-yl) citracon amic acid was placed in a wide dry Pyrex tube which was immersed in an oil bath and provided with a thermometer. The oil bath was heated until fusion of the amic acid then temperature was maintained at ten degrees above the melting point amic acid for (30-45) minutes (10, 11). The fused mixture was poured into a beaker then the resulted solid was purified by recrystallization from a suitable solvent.

2-2- Dehydration By Using Acetic anhydride And Anhydrous Sodium Acetate As Dehydrating Agent:

A mixture of (0.1 mol) of N-(substituted benzothiazol-2-yl) citracon amic acid in (100 mL) of acetic anhydride and (5-10) % by weight of anhydrous sodium acetate was refluxed with stirring for 2hrs. The resulted homogenous solution was cooled to room temperature then poured into excess cold water with vigorous stirring (12). The obtained precipitate was filtered, washed with water , dried and finally purified by recrystallization from a suitable solvent.

Physical properties of the prepared imides [12-24] are shown in Table (2).

3-Microbiological tests

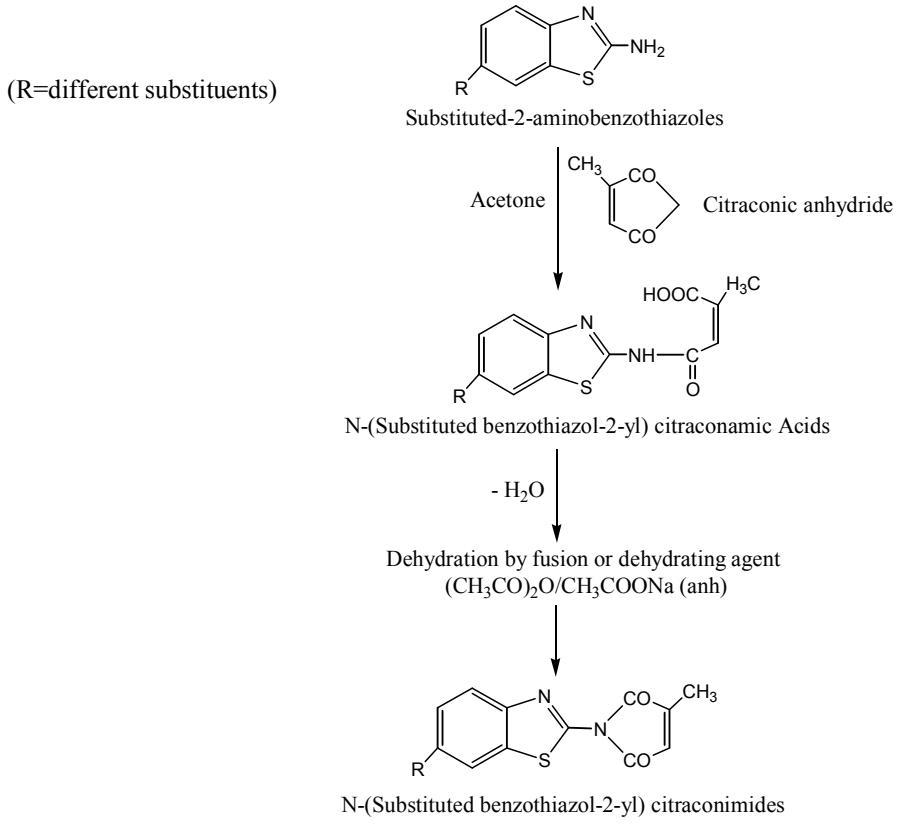
Nutrient agar was added to 1 liter of distilled water in suitable (250 mL) conical flask with stirring and heating until complete dissolving then the flask was Stoppard by cotton and the medium was sterilized in an autoclave for 20 minutes at

(121°C) under pressure of 15 bound/inch. The medium was cooled to (45-55)°C then placed in petridishs about (20 ml) for each one and was left to cool and solidified. The studied bacteria and fungi were placed on the nutrient agar surface using the loop and by streaking processor then the discs saturated with tested compound solutions. Then were incubated for 24 hrs at 37 °C. Inhibition zones caused by the various prepared compounds were determined and the results were listed at Tables (5).

Results and Discussion:

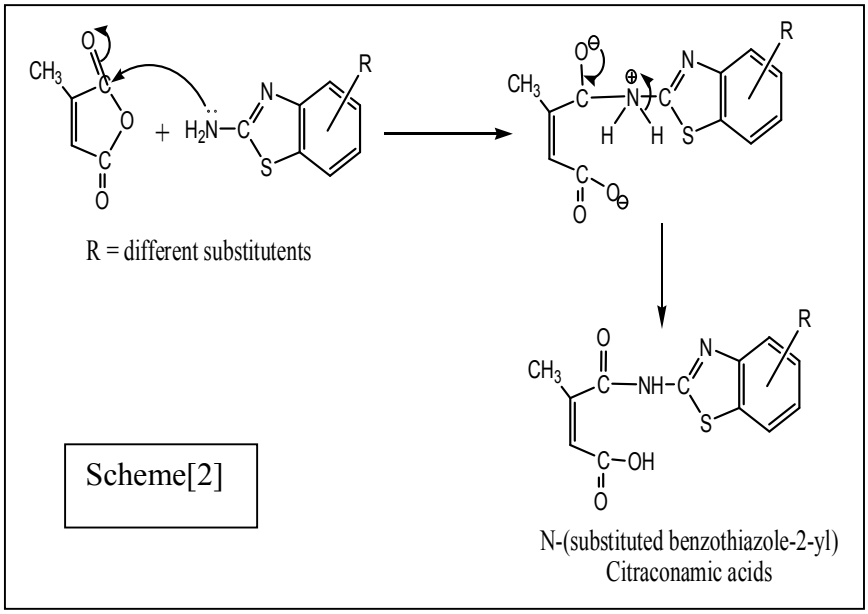
The strategy used in performing this target depended on preparation of primary amine already having benzothiazole moiety in their structures, thus the first step in this strategy involved preparation of twelve 2-aminobenzothiazoles substituted with different substituents by following Thiocyanogen method as reported in literatures (13). The prepared 2-aminobenzothiazoles were introduced in reaction with citraconic anhydride to produce a series of citraconamic acids having benzothiazole moiety in their structures. Dehydration of the resulted citraconamic acids by using dehydrating agents or fusion method afforded the desirable citraconimides.

This linear pathway strategy can be summarized in Scheme



Scheme [1]

Synthesis of citraconamic acids was performed via reaction of equimolar amounts of citraconic anhydride and 2-aminobenzothiazoles (as primary amines). Mechanism of this reaction involved nucleophilic attack of amino group in primary amine on carbon atom of one carbonyl group in citraconic anhydride in scheme [2].



Scheme[2]

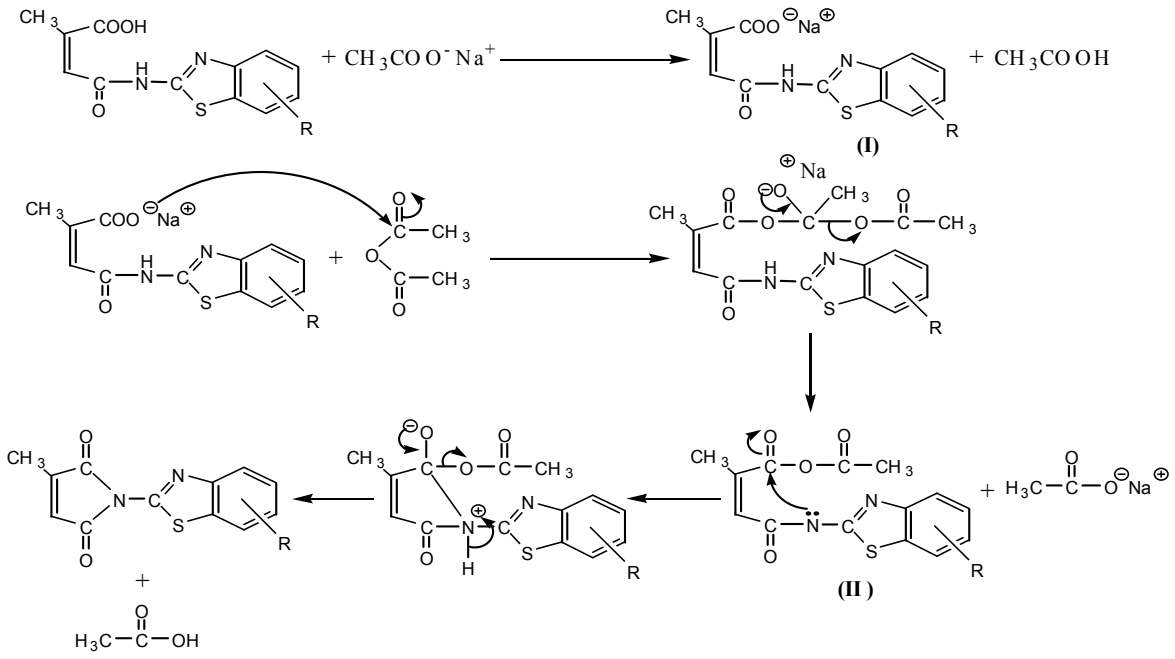
The prepared citracon amic acids are colored solids with sharp melting point and high percent yields. Structures of amic acids [1-12] were confirmed by depending on FTIR and U.V spectroscopy. FTIR spectra of the prepared acids showed characteristic absorption bands at (3270-3463) cm-1 and (3201-3433) cm-1 which were attributed to ν(N-H) amide and ν(O-H) carboxylic respectively(14). Other absorption bands appeared at (1643-1704) cm-1 and (1600-1680) cm-1 which were assigned for ν(C=O) carboxylic and ν(C=O) amide, while ν(C=N) and ν(C-S) for thiazole ring were appeared at (1512-1596) cm-1 and (601-702) cm-1.

On the other hand U.V. spectra of the titled acids showed absorption bands at wave length (245-295) nm and (300-382) nm due to ($\pi\rightarrow\pi^*$) and ($n\rightarrow\pi^*$) transitions in benzothiazole conjugated system and attached citraconamic acid moiety. Also conjugation of substituents in compounds [5, 7, and 11] with conjugated System of molecule shifted the absorptions to longer wavelengths. All the details of FTIR and U.V spectral data of the prepared amicacid are listed in table (3). Finally 1H-NMR spectra of compound [4] showed clear signal at (δ=2.1) ppm belong to CH3 protons and signals at (δ=6 and 6.3) ppm due to 1H vinylic and (N-H) proton respectively

(12). The spectrum also showed signals of aromatic pronots at (δ=7.3 and 7.7) ppm.and singalet signal of OH carboxylic at (δ=11.5) ppm.

C13-NMR of compound [4], N-(6-chlorobenzothiazol-2-yl] citraconamic acid. Showed signal at (20.1 ppm) due to methyl group signals at (117.82-131.3) ppm were due to aromatic carbons, while signals (136.94) ppm belong to carbon atom in thiazole ring. The spectra showed other signals at (146.14) and (149.23) ppm due to two vinylic carbons and other signals at (167.77 and 171.18) ppm due to two carbonyl carbons. he final step in the strategy used in building the desirable cyclic imide involved dehydration of the prepared citraconamic acids.

Dehydration was performed either by fusion technique or by using dehydrating agent.in the presence of anhydrous sodium acetate (15). Mechanism of this reaction involved abstraction of proton from amic acid by the catalyst anhydrous sodium acetate producing (citraconamate ion I) which inturn attacked acetic anhydride producing (citraconamic anhydride II) followed by ring closure(16) as described in equations in Scheme (3).



Scheme [3]

All the prepared citraconimides were colored solids with sharp melting points and were afforded in high percent yields. Structures of imides [13-24] were confirmed by FTIR and U.V spectroscopy. FTIR spectra of the titled compounds showed disappearance of ν(O-H) and ν(N-H) absorption bands indicating success of dehydration reaction. The spectra showed also many clear absorption bands were

shown in citraconimides FTIR spectra including bands at (1712-1720) cm-1, (1512-1581) cm-1 and (1334-1396) cm-1 which were attributed to ν(C=O) imide, ν(C=C) and ν(C-N). Also absorption bands due to ν(C=N) and ν(C-S) for thiazole ring were appeared at (1581-1650) cm-1 and (630-702) cm-1 respectively.

On the other hand U.V. spectra of the prepared imides showed clear absorptions at wave length (272-299) nm and (302-394) nm and at longer wave lengths (411-460) nm in some of them.

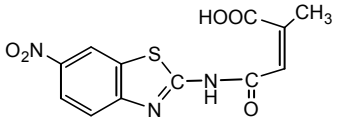
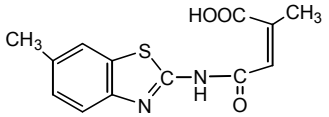
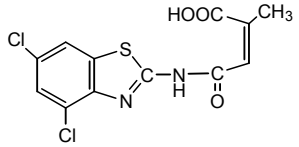
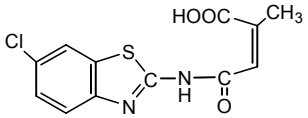
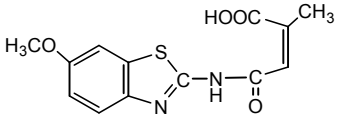
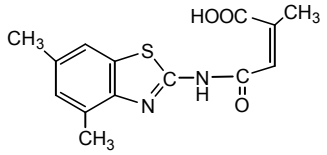
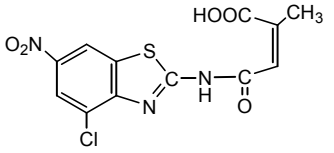
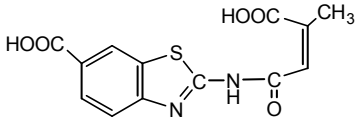
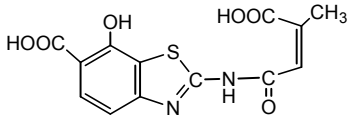
These absorptions were due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in benzothiazole moiety which was in conjugation with Citraconimide moiety (10, 11). Details of FTIR and U.V spectral data of the prepared Citraconimides are listed in table [4]. H-NMR spectra of compound [16] showed signal at (δ=2.1) ppm for CH3 protons and signal at (δ=6.6) ppm for 1H vinylic proton. Signals at (δ=7.4-7.6) ppm belong to aromatic protons. Finally13C-NMR spectra of compound [16],N-(4-chlorobenzothiazole-2-yl) Citraconimide. Showed signal at 9.54 ppm belong to CH3 group. Signals at (119.14-130.95) ppm due to aromatic ring carbons, while signal at (132.67 ppm) due to vinylic carbons and signal at (169.47, 170.44) ppm were due to two carbonyl carbons. Figures (1-12) showed FTIR spectra and U.V. spectra of prepared imides. While Figures (13-16) showed H-NMRand C13 NMR spectra of compounds [4] and [16].\

Biological activity

The prepared compounds were screened for their antibacterial activity against four microorganisms including [Staphylococcus aurous and Streptococcus pyogenes] Types of (Gram positive) bacteria and [E.coli and Pseudomonas aeruginosa]. Types of (Gram negative) bacteria moreover biological activity of the prepared compounds against fungi [Candida albicans].were studied also. The prepared citraconamic acids and citaconimides showed different biological activities against the studied types of bacteria and fungi as shown in table (5). It was noticeable that biological activity of these compounds depend on nature of substituents in their molecules thus compounds (2), (5) and (18) showed high biological activity due to the presence of electron releasing substituents (CH3) and (OCH3) respectively. While compounds [3, 4, 22] which substituted with electron with drawing substituents (Cl) and (COOH) showed slight activity against S.aureus and S.pyogenes But were inactive against other bacteria and fungi. Also compound [13] which were substituted with (NO2) group

showed no activity against S.aureus, S.pyogenes and fungi but slight activity against E.coli and P.aeruginosa.

Table (1): Physical properties of the prepared Citraconamic acids [1-12]

Comp. No.	Compound structure	Color	Melting Points °C	Yield %	Recrystallization Solvent
1		Yellow	141-142	93	Dioxane
2		Faint Yellow	160-162	92	Ethanol
3		white	220 decomp	75	Dioxane
4		Light tan	128-130	92	Methanol
5		Violet	131-132	82	Ethanol
6		Faint gray	150-152	90	Ethanol
7		Orange	158-159	97	Dioxane
8		white	253-254	75	Ethanol
9		Deep Yellow	137-138	50	Acetone

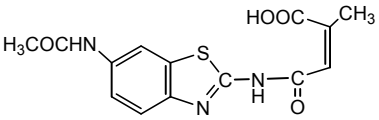
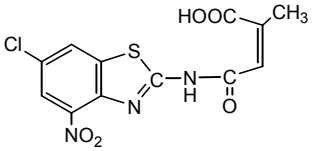
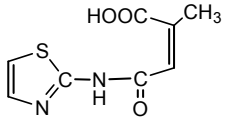
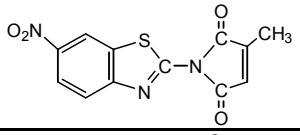
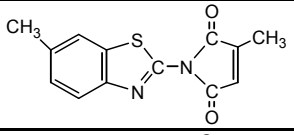
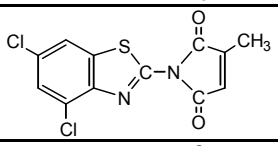
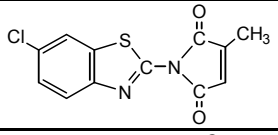
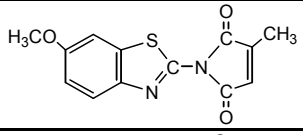
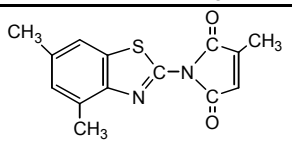
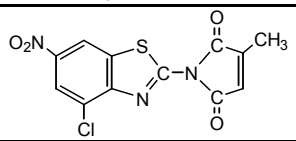
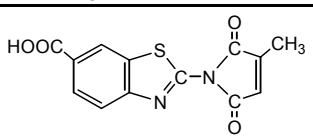
10		Orange	108-110	97	Methanol
11		Faint Yellow	165-166	95	Ethanol
12		Brown	119-120	85	Dioxane

Table (2): Physical properties of the prepared citraconimides [13-24]

Comp. No.	Compound structure	Color	Melting Points °C	Yield %	Recrystallization Solvent
13		Yellow	120-122	91	Petroleum ether
14		Radish Brown	130-132	90	Cyclohexane
15		Paint Brown	97-98	92	Cyclohexane
16		Faint Yellow	75-76	87	Benzene
17		Deep Green	115 decomp	88	Dioxane
18		Faint Brown	68-70	93	Petroleum ether
19		Yellow	108-110	91	Benzene
20		Radish Brown	132-133	89	Acetone

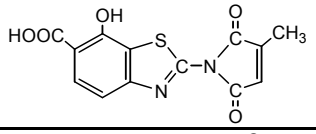
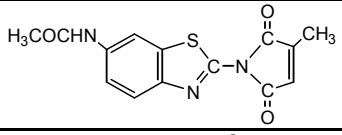
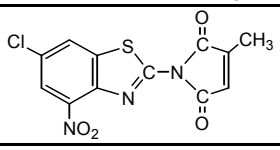
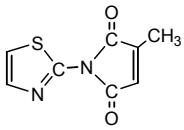
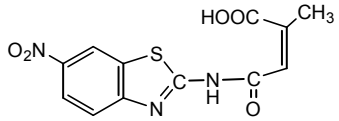
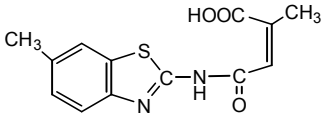
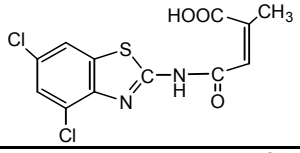
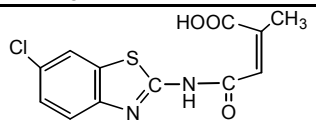
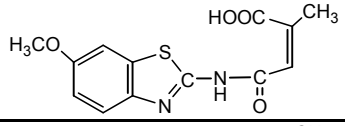
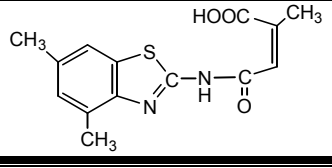
21		faint Brown	96-98	92	Cyclohexane
22		Faint Yellow	78-80	90	Petroleum ether
23		Faint Brown	112-114	86	Cyclohexane
24		Deep Green	70-71	85	Benzene

Table (3): Spectral data of the prepared citraconamic acids

Comp. No.	Compound structure	FTIR spectral data cm^{-1}							U.V. (λ_{max}) nm
		ν(O-H) carboxylic	ν(N-H) amide	ν(C=O) carboxyl	ν(C=O) amide	ν(C=N) thiazole	ν(C-S) thiazole	others	
1		3278	3325	1680	1643	1512	601	ν(NO_2) 1334	345 344 361
2		3286	3325	1643	1600	1566	601	-	301
3		3217	3325	1700	1680	1581	601	ν(C-Cl) 1010	301
4		3201	3278	1704	1635	1535	700	ν(C-Cl) 1087	301
5		3263	3300	1658	1610	1573	671	ν(C-O-C) 1226	301 463
6		3225	3270	1690	1658	1566	678	-	301

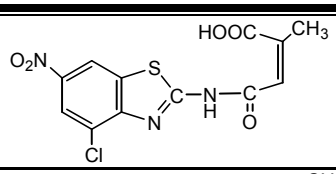
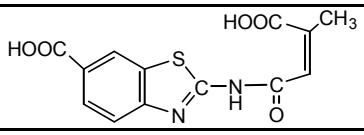
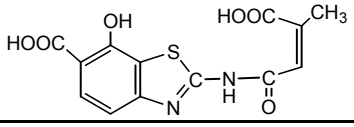
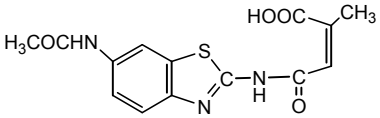
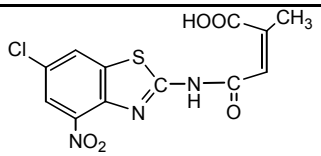
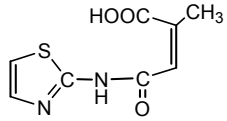
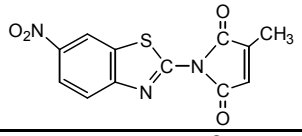
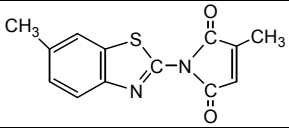
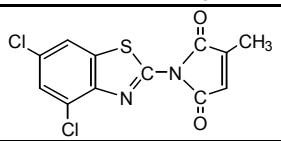
7		3286	3325	1643	1600	1566	601	$\nu(NO_2)$ 1340 ν(C-Cl) 1118	250 419
8		3363	3463	1681	1612	1596	702 632	-	290 335
9		3348	3402	1650	1604	1566	694 617	-	305 364 380
10		3271	3271	1666	1643	1550	655 702	-	295 340 369
11		3425	3425	1643	1643	1558	648	$\nu(NO_2)$ 1342 ν(C-Cl) 1018	300 340 416
12		3433	3433	1681	1650	1566	605	-	300 382 340

Table (4): Spectral data of the prepared citraconimides

Comp. No.	Compound structure	FTIR spectral data cm^{-1}						U.V. (λ_{max}) nm
		ν(C=O) Imide	ν(C=N) thiazole	ν(C=C)	ν(C-N)	ν(C-S) thiazole	others	
13		1765 1720	1650 1519	1519	1342	-	-	345 344 361
14		1712	1610	1542	1410	702	-	301
15		1712	1600	1573	1400	600	ν(C-Cl) 1100	301

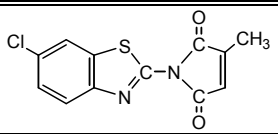
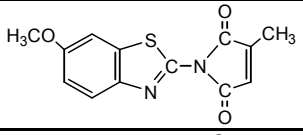
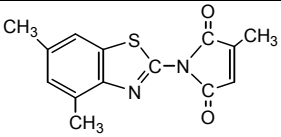
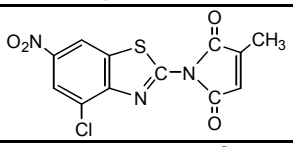
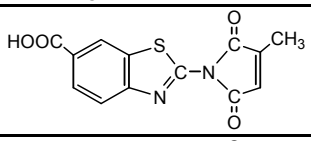
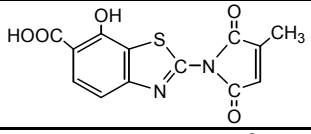
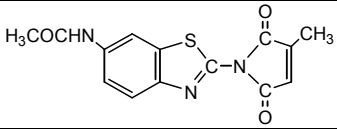
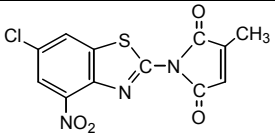
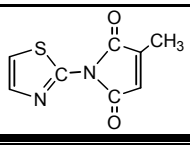
16		1712	1600	1540	1396	-	ν(C-Cl) 1087	301
17		1720	1604	1520	1350	663	ν(C-O-C) 1226	301 463
18		1720	1643	1558	1388	702	-	301
19		1760 1710	1570	1570	1380	672	ν(NO_2) 1326 ν(C-Cl) 1033	250 419
20		1720	1604	1540	1342	-	ν(O-H) 3400	290 335
21		1780 1720	1581	1512	1380	630	ν(O-H) 3250	305 364 380
22		1789 1712	1643	1512	1350	678	ν(N-H) amide 3479	295 340 369
23		1765 1712	1581	1581	1396	694	ν(NO_2) 1342 ν(C-Cl) 1033	300 382 340
24		1712	1615	1533	1365	655	-	300 340 416

Table (5): Antibacterial and antifungal activity of N-(substituted benzothiazole-2-yl) citraconamic acids and citraconimides

Comp. No.	Gram-positive bacteria		Gram-negative bacteria		Fungi
	Staphylococcus aureus	***Streptococcus pyogenes***	***Escherichia coli***	***Pseudomonas aeuroginosa***	***Candida albicans***
2	+++	+++	+	+	+
3	+	+	-	-	-
4	+	+	-	-	-
5	++	++	++	++	+
13	+	+	-	-	-

14	-	-	+	+	-
15	+	+	+	+	+
18	+++	+++	+	+	++
20	+	+	-	-	-
23	+	+	+	-	-

Note: (-) = No inhibition = inactive

(+) = (1-5) mm = weak activity

(++) = (6-10) mm = Moderate activity

(+++) = (11-15) mm = high activity

(++++) = more than (20) mm = very high activity

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