Synthesis, Characterization, Study the Toxicity and Anticancer Activity of N,O-Chitosan Derivatives

MUNA S . Al-rawi

International Journal of Pharmaceutical Research | Apr - Jun 2020 | Vol 12 | Issue 2

Research Article

Synthesis, Characterization, Study the Toxicity and Anticancer Activity of N,O-Chitosan Derivatives

RUWAIDAH S. SAEED, MUNA S. AL-RAWI
Department of Chemistry, College of Education for Pure Science (Ibn Al-Haitham) / University of Baghdad, Iraq.
*Corresponding Author
Email ID: hsnalbyaty009 @gmail.com
Received: 26.12.19, Revised: 21.01.20, Accepted: 29.02.20

ABSTRACT
In the present research, synthesis and characterization of novel heterocyclic compounds substituted chitosan moieties was achieved. Bis Schiff base [1] was utilized as a commencing material for the synthesis method. Schiff base[1] was achieved from the reaction of two mole of 2-amino-5-mercapto-1,3-thiadiazole with one mole of terephthalaldehyde in ethanol absolute. Also, the reaction of compound [1] with chloroacetyl chloride, thioglycolic acid, sodium azide or various anhydrides to give azetidinone [2], thiazolidinone [3], tetrazole [4], and 1,3-oxazepine derivatives [5-7] respectively, then compounds[2-7] were reacted with Na_2CO_3 of distilled H_2O, then chloroacetic acid was added to yield [8-13]. Also, compounds [8-13] reacted with thionyl chloride in benzene to procure [14-19]. *O*- chitosan derivatives[20-25] were synthesized by reaction of chitosan with compounds [8-13] in acidic media in distilled water according to the steps of Fischer and Speier. *O,N* –Chitosan derivatives [26-31] had been synthesized by reaction of chitosan with compounds [14-19] in trichloromethane and pyridine. The synthesized compounds were identified via spectral analysis techniques, including FT-IR, ^{1}H-NMR, UV-Vis Spectroscoy and the elemental analysis (C.H.N-S). Insertion of heterocyclic units to chitosan molecule were expected to improve the selectivity in the potent biological activities, therefore the toxicity study and anticancer activity, were examined for some organic compounds and chitosan derivatives.

Keywords: Azetidinone, Thiazolidinone, Tetrazole , Oxazepine**,** Chitosan derivatives, Anticancer activity, Toxicity study**.**

INTRODUCTION
Chitosan is characterized by its biocompatibility, biodegradability, non-toxicity, antimicrobial, antitumor activities, coagulating activities, bioadhesivity, wound healing capacity, cosmetic industries [(1)], the food industry and waste water treatment, among many other industrial applications medicine and pharmacy[(2,3)], also chitosan in the preservation of agricultural commodities [(4)].In consequence of the poly functionality of chitosan, various kind of modification reactions can be executed on it to achieved different materials with special characteristics, the benefits to mend the properties or gives it new properties, and the backbone of chitosan keep its properties after modification[(5,6)]. In addition to that, chitosan have primary amino groups and increase the number of these groups is associated to the rate of antibacterial activity. Also, the hydroxyl groups at the C6 have obvious activity, by the statute of amino protection[(7)]. Herein, and in continuation of past studies, the synthesis and characterization of some novel heterocyclic compounds substituted chitosan moieties by an easy synthetic are described and examined the toxicity and anticancer activity for them.

EXPERIMENTAL
Materials: All chemicals were supplied from BDH, CDH and SCR.
Instrumentation: FT-IR-Spectra were recorded on a Shimadzu - 8400s in the range (400-4000) cm^{-1} using KBr disk . NMR spectra were analyzed and characterized by (Ultra Shield 500 MHz, VARIAN , Switzerland), and (Ultra Shield 400 MHz, Bruker) , the chemical
shifts are described in ppm as in comparison with the reference TMS. The (C.H.N-S) were performed on an EuroEA Elemental .

Preparation of compounds:

Synthesis of 5,5'-((1,4-phenylenebis (methaneylylidene)) bis (azaneylylidene)) bis(1,3,4-thiadiazole-2-thiol) [1][8]:

Terephthalaldehyde (1.34g, 0.01mol) mixed with (2.66g,0.02mol) 2- amino-5- mercapto -1,3,4 - thiad iazole , EtOH 20mL , with two drops of glacial ac etic acid , refluxing at 70°C for 18h.The reaction m ixture was cooled, the yellow precipitate filtration and recrystallized from ethanol and dried to yield 85%.

Synthesis of 4,4'-(1,4-phenylene) bis (3-chloro-1-(5-mercapto-1,3,4-thiadiazol-2-yl)azetidin-2-one) [2][9]:

mixture solution of (0.02mol) of chloroacetyl chloride / dioxan(10mL) cooled at (0-5) °C, then added (0.02mol) of Et_3N /dioxane(10 mL) and Schiff base[I] (3.64g,0.01mol) /dioxane (10mL) was tardily refluxed in water bath for 12h., cooled and the reaction eeming into ice- water to afford precipitate, then filtered and recrystallization by solution (50benzene : 50ether).

Synthesis of 2,2'-(1,4-phenylene)bis(3-(5-mercapto-1,3,4-thiadiazol-2-yl)thiazolidin-4-one) [3][10]:

Thioglycolic acid (0.02mol) in dry benzene (30mL) was added quietly to (3.64g,0.01mol) of compound[1] .The addendum continuous for 10 min with continued stirring then for about 4 h. , then reflux on a steam bath for 18 h. spare solvent was evaporated and the residue was remedy with $NaHCO_3$, filtered and recrystallized with dioxin.

Synthesis of 5,5'-(1,4-phenylenebis(1H-tetrazole-5,1-diyl))bis(1,3,4-thiadiazole-2-thiol) [4][11]:

To solution of Schiff base[1](3.64g,0.01mol) in DMF 15mL, Sodium azide (0.02mol) was added, the mixture was refluxed for 4h., then it was allowed to cool and the white precipitate was filtered, and recrystallized from petroleum ether.

Synthesis of 1,3-oxazepine derivatives [5-7][12]:

Amixture of (3.64g, 0.01mol) of Schiff base [1] and (0.02mol) of different acid anhydrides (pyromellitic dianhydride , phthalic or maleic anhydride) in dry benzene were reflux for 7h. The crystalline solid was filtered and recrystallized from EtOH.

Synthesis of compounds[8-13][13]:

A liquot (0.01mol) of hot compound [2-7] mixed with (0.04 mol) Na_2CO_3 in (15mL) of distilled H_2O, then (0.02mol) of $ClCH_2COOH$ was added. The solution refluxed for 6h., then added conc. HCl to reached out PH= 2. Filtered the result and washed with H_2O and recrystallized from EtOH.

Synthesis of compounds[14-19][14]:

(0.01mol) of compound [8-13] mixed with $SOCl_2$(0.0 2mol) in dry benzene (15mL) was refluxed for8 h ., the excess of thionyl chloride and benzene were outlying under vacuum.

Synthesis of *O*- chitosan derivatives [20-25] [15]:

(0.01mol) of chitosan was hang in 25 mL of H_2SO_4 (2M) , added (0.01mol) of compounds [8-13] to t his solution .The mixture was refluxing for 8 h an d cooling subsequently. The pH was regulated t o 7 by neutralization with $NaHCO_3$. The compound was precipitated in aceton , filtered and washed wi th acetone to remove the unreacted acid and then dried at 60°C in oven for24hrs.

Synthesis of *O*,N chitosan derivatives [26-31][16]:

(0.01mol) of chitosan was marinated in 50 mL $CHCl_3$ and pyridine (1:1) for 20h. , (0.01mol) of compounds[14-19] was added to them under water –ice bath, the mixture was stirred at 100^0C for 14h., cooled and poured into 50mL MeOH, after cooled at 4^0C, then filtered. The precipitate was rinsed with MeOH and dried at 50^0C.

RESULTS AND DISCUSSION

The synthesis of new derivatives starting from bis Schiff bases is evinced in Scheme (1).

Compound [1] was synthesized through the reactio n of 2-amino-5- mercapto-1,3,4- thiadiazole with terephthalaldehyde in ethanol under reflux for 18h. The FT-IR of compound [1] showed the appearance bands at (2357) , (1616,1645) and (1554,1568) cm^{-1} due to SH group, (C=N) exocyclic and (C=N) endocyclic, respectively.

The Schiff base[1] was treated with $ClCH_2COOCl$ and Et_3N. The FT-IR of compound [2], exhibited the presence of absorption in region (1707) cm^{-1} and (850) cm^{-1} refer to stretch vibration of (C=O) group of azetidine ring and C-Cl, respectively.

Compound [3] was synthesized through the reaction of compound[1] with thioglycolic acid in dry benzene. FT-IR for compound [3] showed the occurrence band at (1708)cm^{-1} due to C=O group of thiazolidinone ring. Tetrazole derivative [4] synthesized by reaction of schiff base[1] with NaN_3 in DMF. FTIR of compound [4] display demise of absorption of imine group with appearance of new

absorption in the region (1525, 1386) cm^{-1} which are assigned to N=N and C-N stretching.

A new 1,3-oxazepine derivatives [5-7] were prepared from substituted imine [1] with different anhydrides: pyromellitic dianhydride , phthalic anhydride , or maleic anhydride, scheme(2). FTIR of compound [6] , exhibited appearance of band at (1670 , 1710) cm^{-1} for carbonyl groups in oxazepine ring[17] and bands around (1259 and 1143 cm^{-1}) belong to asy. and sym. (C-O-C) band. The ^{1}HNMR(DMSO-d_6) of compound[6] showed: a singlet signal with a chemical shift region at *δ* 10.14ppm caused by the presence of N-CH proton and a singlet signal with a chemical shift region at *δ* 8.84 ppm caused by the presence of SH protons, finally, twelve aromatic protons were ranged from δ 7.36-8.20ppm.

Compounds[8-13] were prepared in basic media, by the reaction compounds [2-7] with chloroacetic acid in distilled water. FTIR spectrum of compound [12] establishs a bands at (3400-2400) cm^{-1} for hydroxyl group and (1693) cm^{-1} for carboxylic group . The ^{1}H-NMR exhibited a broad singlet signal with a chemical shift at *δ* 13.14 ppm as a result of the two protons of carboxylic protons, additional signal at δ10.07 ppm due to the presence of two protons for CH-N, multiple peaks appreared at δ7.33-8.89 ppm for aromatic protons and a singlet signal at δ 3.91 for four protons for S-CH_2.

The condensation reaction of synthesized compounds [8-13] with $SOCl_2$ using a dry benzene as solvent resulted derivatives [14-19] with a good yield. FT-IR of compound [18], interpreted the disappear the band at 3400-2400 cm^{-1}due to (OH)group of carboxylic acid and appearance of band at 1759 cm^{-1} due to acyl chloride. The Elemental analysis (C.H.N-S) data of these compoundes are listed in Table(6), and they are conform with the suggested theoretical formulas for them .

The newly *O*- chitosan derivatives [20-25] were prepared through the reaction between [8-13]with chitosan in distilled H_2O in acidic media, scheme (3). FT-IR of derivative [25] interpreted the presence of a big band at (3400) cm^{-1} refer to the stretch vibration of O-H and N-H from the intra- and extra-molecular hydrogen bonding of chitosan molecules and a new absorption band at 1711 cm^{-1} due to C=O of ester. ^{1}HNMR of compound [25] elucidated singlet signal with a chemical shift region at δ 8.24-8.06ppm due to the presence of protons of hydroxyl groups of chitosan, a multiple signals at δ6.98-7.89ppm for aromatic protons, singlet signal at δ6.22 ppm as a result of the presence of two protons for N-CH groups. Also, the characteristic region at 4.72-5.23ppm corresponded to the non-anomeric proton (H-1, H-3, H-4, H-5and H-6) of chitosan, doublet of doublet at δ3.64 -3.50ppm for proton CH=CH, singlet signal at δ3.73ppm for proton of SCH_2 groups and signal at 2.87ppm due to CH_2O singlet signal at δ2.07ppm for two protons of NH_2 group[15]

O,N- chitosan derivatives [26-31]were prepared through the reaction between [14-19]with chitosan in trichloromethane and pyridine as a solvent, scheme(3). FT-IR of compound [30] illustrated a large peak at 3363cm^{-1} is related to the stretching of O-H and N-H from the intra-and intermolecular hydrogen bonding of chitosan molecules and appearance of new band at 1726, 1666 cm^{-1} due to carbonyl group of ester and amide groups, respectively. ^{1}HNMR spectrum of compound [30] elucidated singlet signal with a chemical shift region at δ 9.14 ppm due to the presence of protons of NHC=O group, singlet signals at δ 8.14-8.16ppm for OH groups of chitosan, a multiple signals at δ7,49-7.76ppm for aromatic protons, a sharp signal at δ7.31 ppm for proton CH-N. Also, the characteristic region at δ 4.24-4.65ppm corresponded to the non-anomeric proton (H-1, H-3, H-4, H-5and H-6) of chitosan, singlet signal at δ4.66ppm for eight proton of SCH_2 groups and signal at δ 2.54ppm due to CH_2O, singlet signal at δ1.29ppm for two protons of NH_2 group[15,16].

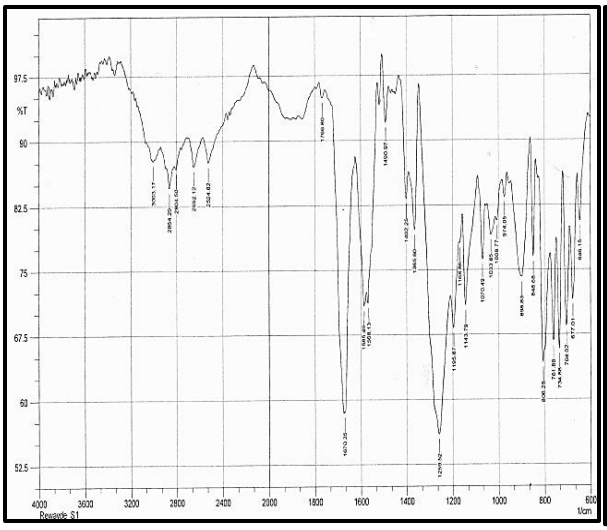


Fig.1: FTIR spectrum of compound [6]

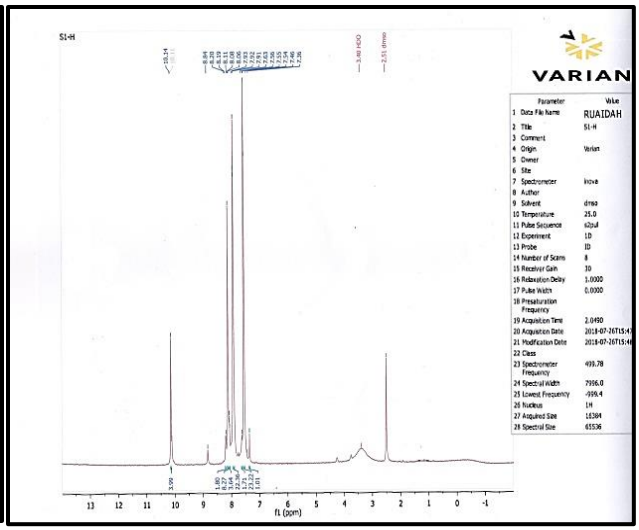


Fig.2 : 1H-NMR spectrum of compound of [6]

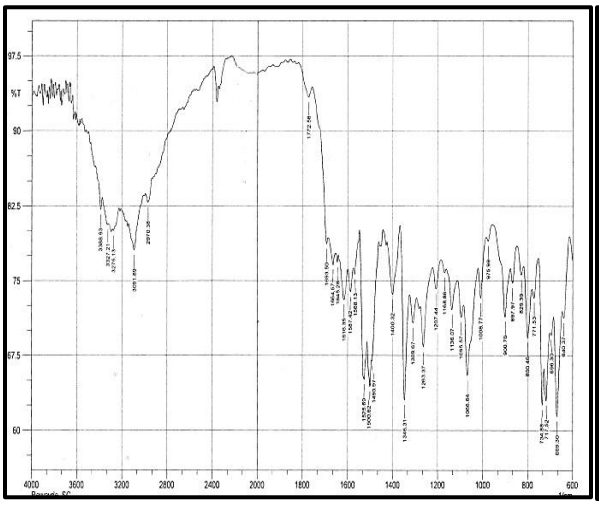


Fig.3: FTIR spectrum of compound [12]

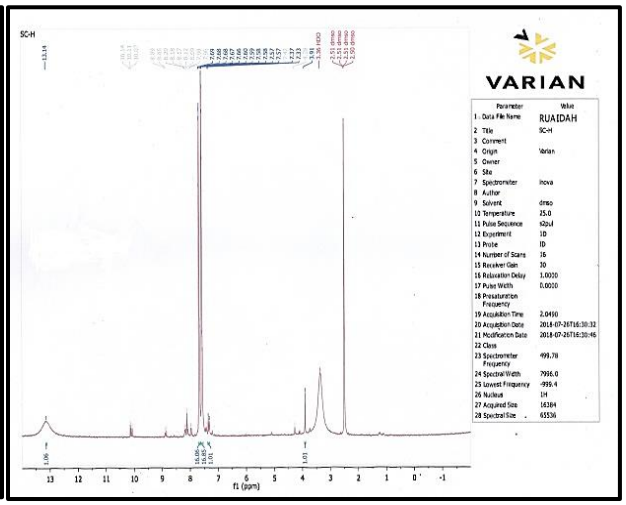


Fig.4: 1H-NMR spectrum of compound of [12]

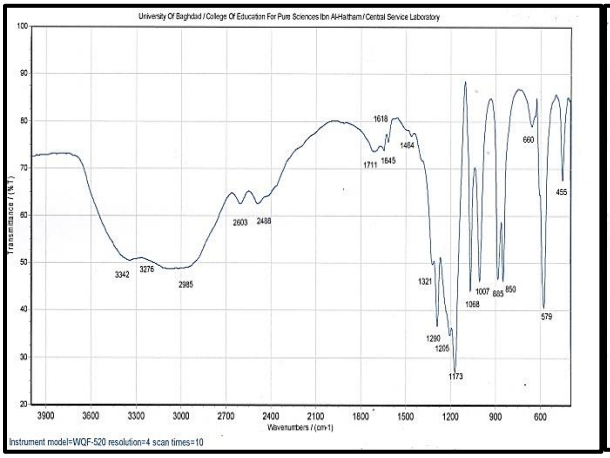


Fig.5: FTIR spectrum of compound [25]

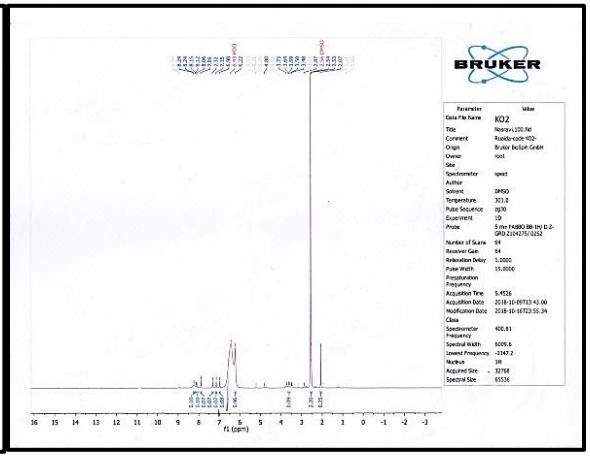


Fig.6 : 1H-NMR spectrum of compound of [25]

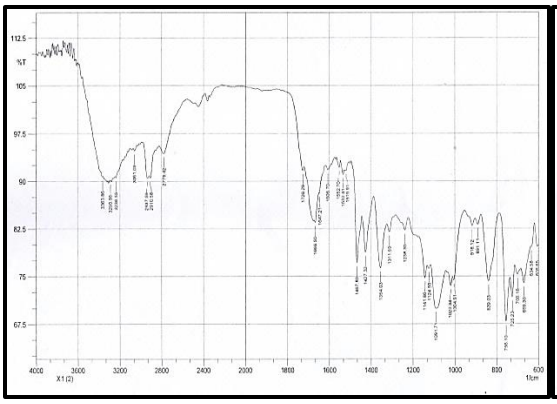


Fig.7: FTIR spectrum of compound [30]

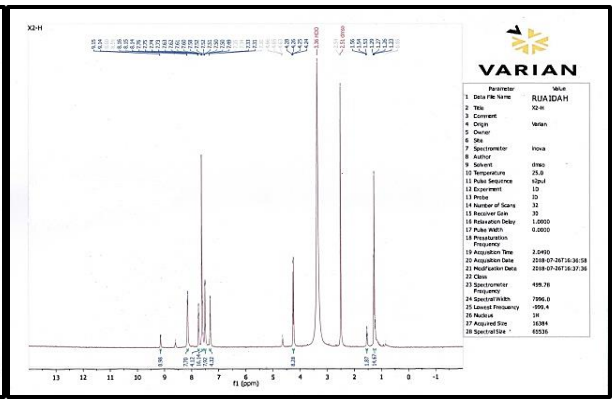


Fig.8 : ^{1}H-NMR spectrum of compound of [30]

Table 1: FT-IR spectroscopy data of of compounds [5-7]

Comp. No.	(S-H) cm^{-1}	(C=O)N	(C=O)O	(C=N) of thiadiazole	(C-S)
[5]	2355	1693	1726	1608	673
[6]	2355	1670	1710	1610	677
[7]	2357	1697	1720	1631	683

Table 2: FT-IR spectroscopy data of compounds[8-13]

Comp. No.	(O-H) cm^{-1}	(C-H) arom. cm^{-1}	(C=O) carboxlic cm^{-1}	(C=N) of thiadiazole	(C=C) cm^{-1}
[8]	3400-2400	3050	1693	1608	1590
[9]	3400-2400	3043	1699	1627	1595
[10]	3300-2400	3059	1697	1637	1597
[11]	3400-2400	3057	1695	1620	1598
[12]	3400-2400	3055	1693	1616	1587
[13]	1400-2400	3053	1678	1618	1580

Table 3: FT-IR spectroscopy data of compounds [14-19]

Comp. No.	(C-H) arom. cm^{-1}	(C=O)-Cl	(C=N)	(C=C)
[14]	3084	1755	1610	1579
[15]	3047	1770	1604	1591
[16]	3007	1760	1608	1588
[17]	3010	1766	1637	1581
[18]	3057	1759	1630	1597
[19]	3020	1760	1627	1590

Table 4: FT-IR data of polymers[20-25]

Com. No.	(O-H) and (N-H) cm^{-1}	(C-H) aliph.	(C=O) ester.	($-CH_2-O-CO$)	(C=C)	(C-O-C)
[20]	3257	2974-2926	1716	1259	1590	1091
[21]	3255	2970-2895	1712	1263	1558	1093
[22]	3400	2943-2916	1712	1288	1587	1066
[23]	3398	2926-2858	1724	1286	1591	1068
[24]	3226	2941-2910	1716	1280	1587	1066
[25]	3342	2945-2904	1711	1290	1597	1068

Table 5: FT-IR data of polymers[26-31]

Com. No.	(O-H) and (N-H) cm^{-1}	(C-H) aliph. cm^{-1}	(C=O) ester.cm^{-1}	(C=O) amide cm^{-1}	(C=C) cm^{-1}	(-CH_2-O-CO) cm^{-1}	(C-O-C) cm^{-1}
[26]	3394	2931-2872	1714	1631	1570	1271	1112
[27]	3381	2943-2890	1722	1645	1590	1290	1090
[28]	3358	2924-2854	1712	1662	1585	1240	1062
[29]	3329	2924-2856	1712	1637	1595	1273	1072
[30]	3363	2937-2910	1726	1666	1575	1238	1091
[31]	3219	2920-2850	1718	1664	1587	1233	1066

Table 6: The Elemental analysis of derivatives[1-13]

Com. No.	Theoretical				Experimental			
	C%	H%	N%	S%	C%	H%	N%	S%
[1]	39.56	2.19	23.07	35.16	39.99	2.38	23.62	35.49
[2]	37.13	1.93	16.24	24.75	37.53	1.68	16.64	24.81
[3]	37.50	2.34	16.40	37.50	37.79	2.12	17.1	37.71
[4]	32.28	1.34	37.66	28.69	32.57	1.64	37.82	28.88
[5]	48.00	1.50	10.50	16.00	48.42	1.76	10.81	16.23
[6]	50.90	2.42	12.72	19.39	51.21	2.54	12.82	19.67
[7]	42.85	2.14	15.00	22.85	42.99	2.29	15.33	22.98
[8]	37.91	2.21	13.37	20.22	37.99	2.42	13.50	20.39
[9]	38.21	2.54	13.37	30.57	38.32	2.65	13.65	30.66
[10]	34.16	1.77	29.89	22.77	34.45	1.92	30.08	22.92
[11]	47.16	1.74	9.17	13.97	47.56	1.86	9.32	14.02
[12]	49.48	2.57	10.82	16.49	49.53	2.69	10.97	16.63
[13]	42.60	2.36	12.42	18.93	42.76	2.44	12.80	19.01

Anticancer activity

Preparation of cell lines for cytotoxicity assay [18] by using cultured cells (96wells) in micro titer plate. The absorbance was measured at(620 nm) on a micro plate reader. Calculated cell growth inhibition rate granted to equations[19]:

$$\text{Inhibition rate} = \frac{mean\ of\ control - mean\ of\ treatment}{mean\ of\ control} \times 100$$

Twelve of prepared compounds were examend for their anticancer activity. One cell lines were used human breast carcinoma cells(MCF-7)cell line described by Freshney. All compounds modifier of chitosan [(20-22), (29-31)] exhibit good inhibition compare with organic compounds[(8-10),(17-19)] befor modification. Also all derivatives except [20,21] showed more than 50% inhibition for MCF-7 cell line. We need to further inspection to know mechanism by which the heterocyclic unit act to give a potent cytotoxic effect that might get the chitosan derivatives an interest for being promise anticancer drugs.

Table 7: Anticancer activity of some synthesized compounds.

Comp.No.	inhibition of cells growth for MCF-7	Comp.No.	inhibition of cells growth for MCF-7
[8]	30.2%	[20]	57.4%
[9]	12.9%	[21]	21.4%
[10]	0%	[22]	5.4%
[17]	41.7%	[29]	65.3%
[18]	50.0%	[30]	54.9%
[19]	43.1%	[31]	53.6%

Acute Toxicity Test[20,21]

distilled water and. Post treatment, the mice have been fed, and after that, their weights were registered followed by observing for general toxicity symptoms signs, mortality, and behavior for 14 days. In this study The mice behaviors were recorded, over the next 14 days. Moreover, some mice have been sacrificed with cervical dislocation and liver, kidneys, and heart have been weighed

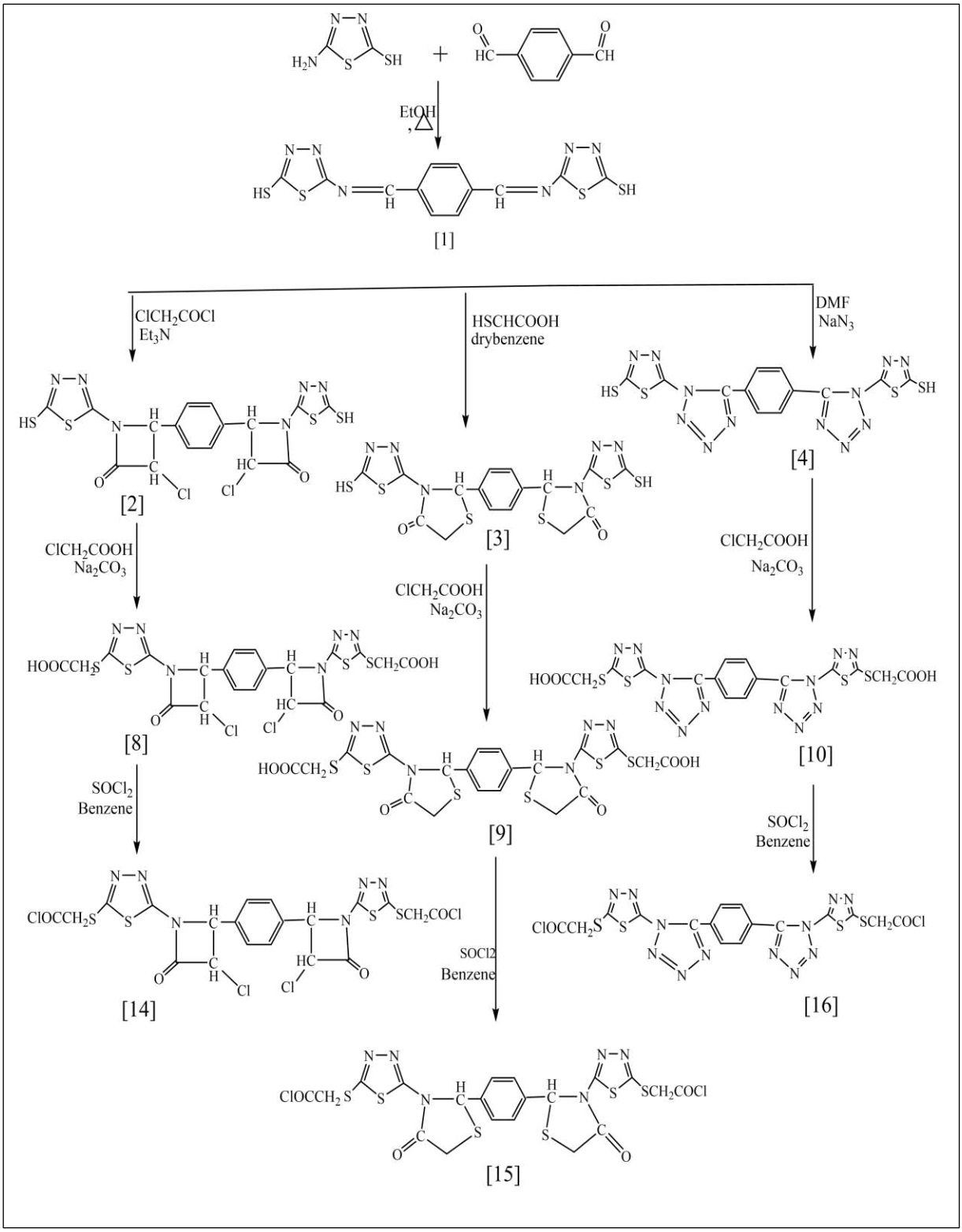
Three groups of 45 albino mice had applied to estimate the acute toxicity of some synthesized compounds (20 , 21 , 29, 30, 31), using the Lorke-written method. Mice were fasted for 18 h with free access to water and food before test. The compounds were dissolved in distilled water and treated through injection (5g/kg and 10g/kg). The treatment group and the control group were compared with doses of the injection and the study showed after 14 days: no mortality with doses 5 and 10 g/kg body weight, no contrast in the weight of the mice daily measured between the group control and the treated groups, no modification in mice behaviors was carried out, and no toxicity symptoms were reported. Moreover, some mice have been sacrificed with cervical dislocation and liver, kidneys, and heart have been weighed. The visual evaluation of organs of mice showed normal appearance.

CONCLUSION

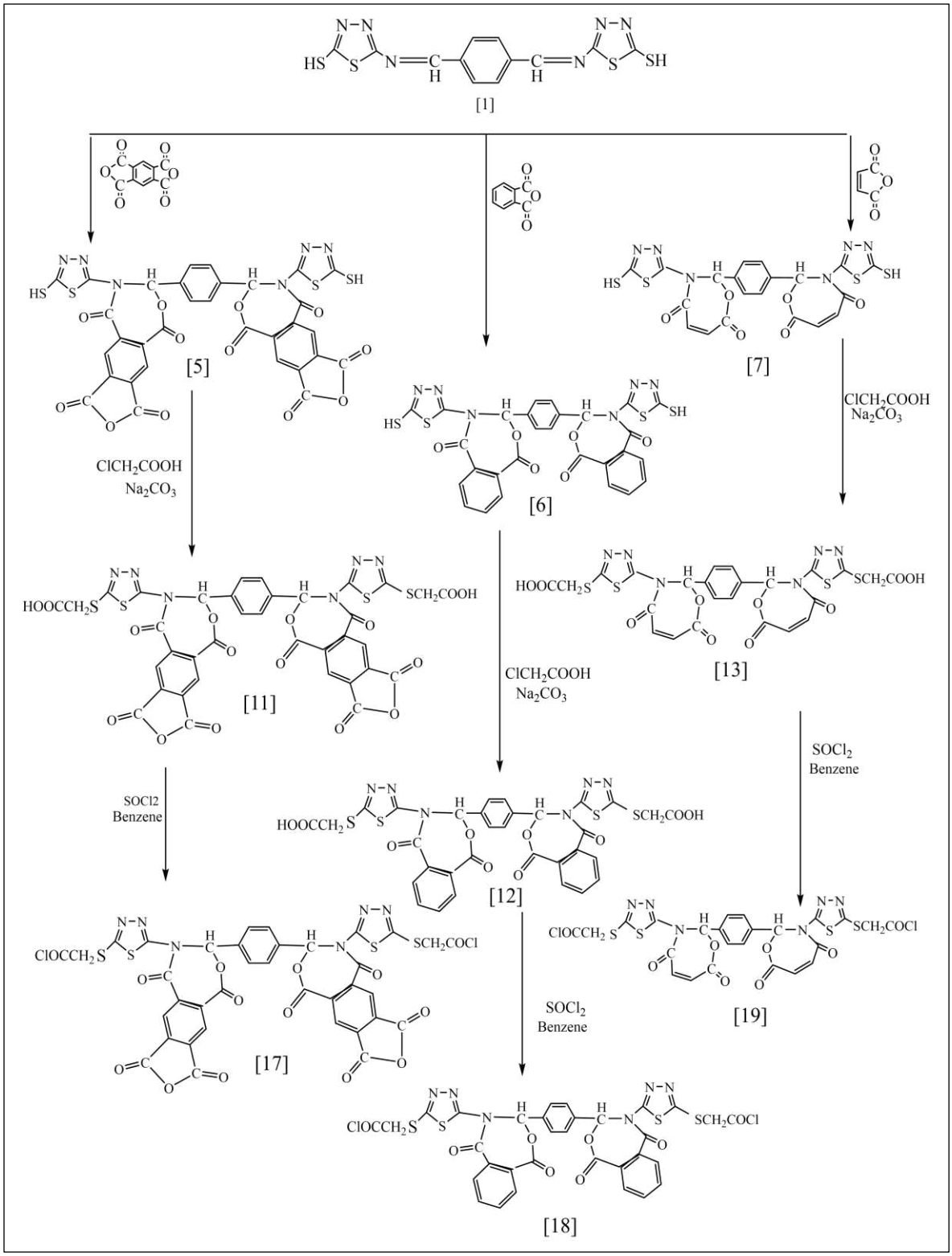
In this research, novel substituted heterocyclic derivatives from chitosan have been synthesized and characterized. The synthesized compounds were identified via spectral analysis techniques, including the elemental analysis (CHN-S). The newly synthesized compounds are expected to be significant for additional research-based therapeutic potency of chitosan as bioactive moiety through structural modifications.

REFERENCES

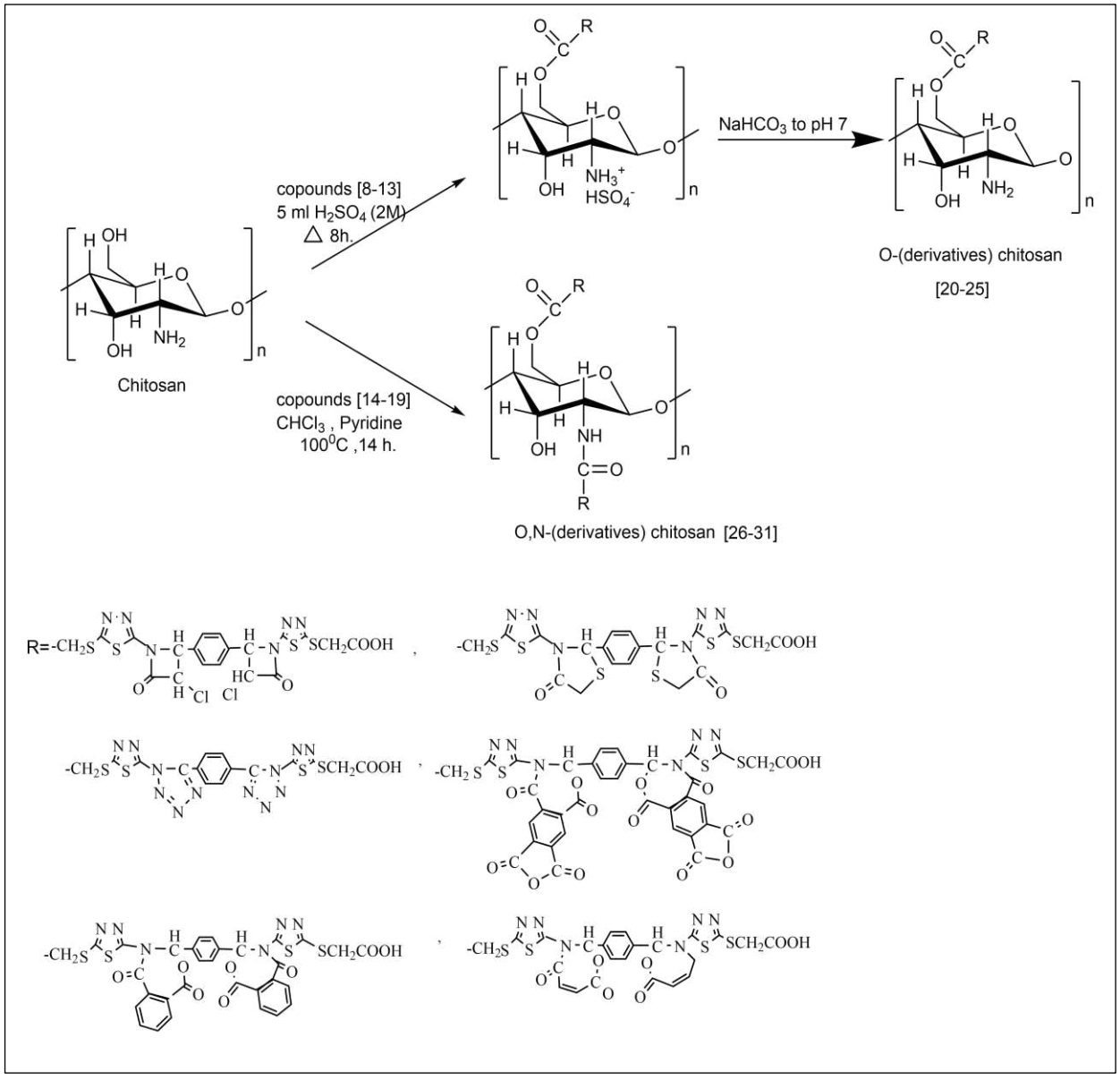
1. Maha A.Y. and Husham A. S. , Journal of Global Pharma Technology , 2019 11, Issue 02 (Suppl.) : 392-398
2. 2.Rayane S.A. , Bianca R. F. ,Vítor A. L. , Raid Í. R. , Eunice P.N. L. , Rodrigo J. S. L. , Carlos A.P.C. and Marcus Vi.L. F. , Mar. Drugs , 2017; 15: 141
3. Ahmed, T. A. ; Aljaeid , B.M. , Drug Des. Dev. Ther. , 2016 ; 10 :483–507.
4. Bautista-Banos S. ; Romanazzi G.; Jiménez-Aparicio A. (Eds.) Chitosan in the Preservation of Agricultural Commodities; Academic Press, Elsevier: Amsterdam, The Netherlands, 2016
5. Mourya V.K , Inamdar N.N. Reactive and Functional Polymers (2008); 68: 1013-1051
6. 6.Mahmoud A. A. , Ayat A. A. and Fadhel S. M., *Ibn Al-Haitham Jour. for Pure & Appl. Sci.,* 2016; 29 (2)
7. Wang J., Wang H., Int. J. Biol. Macromol. (2011);48:523-529
8. Mahmoud M.J.,Al-Rubaiy Z.M.,Al-Kubaisy R.K., Al-Najafi M..M. ,Al-Jumaily H.M:*Ibn* 8.
9. *Al-Haitham J.for pure and appl.sci*. , 2004 ; 17(1):103-110
10. 9-Ali H. S. , Khalid F. A. , Ruwaidah S. S. , *Ibn Al-Haitham Jour. for Pure & Appl. Sci.,* (2014) ; 27 (3) : 350-364.
11. Ruwaidah S. S. , Fadhel S. M. and Ali H. S. , J. Pharm. Sci. & Res. 2019 ; 11(3): 733-740
12. Al-Rawi M.S. , Hussei D.F. ,Al-Taie A.F. ,Al-Halbosiy M.M. and Hameed B.A. ,Journal of physics : Conf .Series 1003(2018)012012
13. Zainab A. S. , Hasan Th. Gh. , Iraqi Journal of Science, 2018 ; 59(1A) : 1-8
14. Shrba H.A. , Hassan A.H., Ali F.K., Inter J. for Sci. and Technology, (2013) ; 8(2): 55-61.
15. 14-Fouad M.S, Redha I., Al-Bagat , Araa Al – Juboori , Al- Mustansiriya J. Sci. , (2006) ;17(3):15-26.
16. 15.Badawy M .E.I.and Rabea E.I. , African Journal of Microbiology Research , (2013) ;7(20) :2259-2268.
17. 16.Zhimei Z. , Bayaer A. , Hui X. and Shuang Z. , International Journal of Biological Macromolecules ,(2012) ; 50 :1169-1174.
18. 17.Eman M. H. and Hussam Z. N. , *Ibn Al-Haitham Jour. for Pure & Appl. Sci* , 2018; 31(1) .
19. 18.Freshney RI "Culture of Animal Cells: A manual of Basic Technique and Specialized Applications," 6th Edition, Wiley: New York ,(2010).
20. 19.Gao S. , Ya BP. , Dong WG. and Luo HS. "Ant proliferative effect of octreotide on gastric cancer cells mediated by inhibition of Akt/PKB and telomerase," World J. Gastroenterology. (2003) ; 9 (10):2362- 2365.
21. 20.Hanane M. , Hindawi, Advance in pharmacological science , 2018 : 3287193.
22. Aké-Assi E. , N'guessan K. and Kouassi AF, European Scientific Journal , 2015;11 (27) : 92–100.


EtOH
[1]
ClCH2COCl
Et3N
HSCHCOOH
drybenzene
DMF
NaN3
[2]
[3]
[4]
ClCH2COOH
Na2CO3
ClCH2COOH
Na2CO3
ClCH2COOH
Na2CO3
[8]
[9]
[10]
SOCl2
Benzene
SOCl2
Benzene
SOCl2
Benzene
[14]
[15]
[16]

Scheme 1



Scheme 2


Chitosan
copounds [8-13]
5 ml H_2SO_4 (2M)
△ 8h.
$NaHCO_3$ to pH 7
O-(derivatives) chitosan
[20-25]
copounds [14-19]
$CHCl_3$, Pyridine
100^0C ,14 h.
O,N-(derivatives) chitosan [26-31]
R=-CH_2S
SCH$_2$COOH

Scheme 3