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Synthesis, Characterization and Antimicrobial Activityof 3-substituted Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole

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Abstract:

New series of tricyclic benzo[d]thiazole derivatives (2-12) incorporated into fused to different five membered nitrogen and sulphur containing heterocyclic were prepared from 2-hydrazinobenzo[d]thiazole (1) when treated with triethylformate, acetic anhydride, ethyl chloro acetate, carbon disulphide in alkali, and urea respectively.

Compound(1) converted to Schiff's bases by the condensation different aromatic aldehydes, the synthesized Schiff's bases were cyclized by bromine in acetic acid to form triazole ring in the new derivatives (7-11) , which might result in biologically active agents. Similar new tricyclic compounds, triazolobenzothiazol-3-amine [12], was obtained from action of benzo[d]thiazole-2-thiol with thiosemicarbazide. The structures of the new compounds have been characterized by elemental analysis and spectral data.

Newly synthesized compounds (2-12) were screened for their antibacterial activity against four bacterial species. They were found to exhibit good antibacterial activity.

Keywords: *3-substituted Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole, 2-hydrazinobenzo[d]thiazole,benzo[d]thiazole-2-thiol, antibacterial activity*

الخلاصة:

سلسلة جديدة من ثلاثية الحلقات غير المتجانسة مشتقة من بنزو [د]ثايزول (2-12) تحوي على ذرات مختلفة من النيتروجين والكبريت تم تحضيرها عند معاملة المركب 2- هيدرازينو بنزو [د] ثايزول (1) مع ثلاثي اثيل فورمات، أنهيدريد الخلات،كلورو خلات الاثيل، ثنائي كبريتيد الكربون في محلول القلوي و اليوريا؛ على التوالي (2-6)، كذلك حُول المركب (1) الى قواعد شف بتكاثفه مع الديهايدات اروماتية مختلفة.

ثم تم اجراء الغلق الحلقي لقواعد شف المحضرة بمعاملتها مع محلول البروم في حامض الخليك لتكوين المشتقات الجديدة(7-11) التي تحتوي على حلقة الترايازول والتي قد تستخدم كمواد فعالة بايولوجياً.

اخيراً تم تحضيرمركب جديد ثلاثي الحلقات (12)، تم الحصول عليه من تفاعل بنزو [د] ثيازول - 2 - ثيول مع ثايوسيمي كاربازايد.

شخصت المركبات الجديدة عن طريق التحليل الأولي والبيانات الطيفية. تم دراسة الفعالية البايولوجية لبعض المركبات المحضرة الجديدة (2-12) ضد أربعة انواع من البكتريا وقد اظهرت نشاط مضاد للجراثيم جيدة.

Introduction:

The chemistry and pharmacology of benzothiazole derivatives have been of great interest because of its various biological activity [1,2]. The benzothiazole have received the attention of medicinal chemists due to their wide range of biological activities which include anti-inflammatory, analgesic, antibacterial activities [3,4].

The aim of the present work to synthesis new series tricyclic compounds fused to different five member nitrogen and sulphur containing heterocyclic derivative of 2-hydrazino-1,3-benzo-thiazole and benzo [d] thiazole -2- thiol and studies their antibacterial activities of

some synthesized. Hence, in the present study, the 2nd position in benzo[d]thiazole-2-thiol moiety having thiol (-SH) group, was used as the target for the chemical modification.

Materials and Methods:

Apparatus and Chemicals

All reagents and solvents used were of Merck, Fluka and BDH.The melting points were determined in open capillaries tube on Stuart SMP10 Melting point apparatus. The purity of the compounds was confirmed by TLC using silica gel (0.5mm thickness, Merk) and visualized in iodine. The IR spectra were recorded in potassium bromide on Shimadzu FTIR-8400S. The elemental analyses (C, H, and N) were performed using Perkin-Elmer 240 C analyzer. Their results were found to be in good agreement with calculated values.

Experimental

2-hydrazinobenzo[d]thiazole [5, 6]. (1)

To a stirred solution of benzo[d] thiazole-2-thiol (0.1mol) in (25 ml) ethanol solution(0.1mole) of hydrazine hydrate (0.13 mol, 80%) was added drop-wise with continuous stirring at 65°C.the mixture was poured on crushed ice; the solid product was filtered, dried and recrystallized from ethanol.

Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole [7]. (2)

A mixture of (1) (0.33 g, 0.002 mol), triethylformate (0.29ml, 0.002mol) in (20 ml) absolute ethanol was refluxed for 8 hr. The solvent was evaporated under reduced pressure. The solid obtained was filtered, recrystallized from ethanol.

Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole-3-methyl [7]. (3)

A mixture of (1) (0.33 g, 0.002 mol), acetic anhydride (0.2 ml, 0.002 mol) in (20 ml) absolute ethanol was refluxed for 6 hr. The mixture was cooled and poured onto ice/cold water with few drops of HCl acid. The solid obtained was filtered, recrystallized from ethanol.

Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole-3-(2H)-one [7] (4)

A mixture of (1) (0.33 g, 0.002 mol) and (0.2 ml, 0.002 mol) of ethyl chloroformate in pyridine (10 ml) was refluxed for 8 hr. The resultant mixture was cooled and poured onto ice/cold water with few drops of HCl acid. The formed precipitate was filtered off, dried purified by recrystallization from ethanol.

Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole-3-thiol [8]. (5)

A mixture of (0.152g, 0.002 mol) carbon disulphide, ethanol (60 ml), compound (1) (0.29 g, 0.0018 mol) and (0.112 g 0.002 mol) potassium hydroxide in (5 ml) water was refluxed on water bath for two hours. Ethanol was removed and the residue was dissolved in an aqueous (20 ml, 5%) potassium hydroxide. The solution was filtered and the filtrate was acidified with dilute hydrochloric acid and again filtered. The solid thus obtained was recrystalized with ethanol.

Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole-3-ol [8]. (6)

Compound (1) (0.50g, 0.003 mole) was heated with pre-dried urea (0.4g, 0.0066 mole) at 180-190°C for 6 hr on oil bath .Then the contents were cooled and NaOH (30 ml, 5%) was added to it. The solution was filtered and acidified with dilute HCl. thus obtained solution was filtered, dried and purified by recrystal-lization from absolute ethanol.

3-arylbenzo [4, 5]thiazolo[2,3-c] [1,2,4] triazol (7-11)

A mixture of compound (1) (3.304 g, 0.02 mole) and appropriate aromatic aldehyde (0.02 mole) and 2-3 drops of glacial acetic acid in benzene (40 ml) was refluxed on water bath for 1 hr. it was cooled, filtered, dried and recrystallised from ethanol[6].

Schiff s base compounds (0.005 mole) were suspended in glacial acetic acid (5 ml) and anhydrous sodium acetate (0.12g) was added. To this stirred mixture a solution of bromine (0.1g) in glacial

acetic acid (2 ml) was added drop-wise. The solution was poured into water (50 ml).the separated crystals were collected, washed with water dried and recrystallized from ethyl acetate[5].

Benzo[4,5]thiazolo[2,3-c][1,2,4]triazole-3-amine [9]. (12)

A mixture of benzo[d]thiazole-2-thiol (0.167 g, 0.001mol) and (0.9 g, 0.001mol) thiosemicarbazide in ethanol (25 ml) was refluxed for 6 hr. a positive test for evolving of hydrogen sulfide by lead acetate stripe was noticed during the refluxing process, the solvent was evaporated and residue purified by recrystallization from ethanol.

Biological Screening

Compounds (2-12) were screened for their antibacterial activity against bacterial species *Klebsiella sp., E. coli. Enterococcus sp., Lactobacillus sp by* agar well-diffusion method[10, 11]. using Mueller Hinton agar medium to assess the activity of the chosen synthezied compounds. The drug Streptomycine was tested under similar conditions for comparison. Wells were made (by scooping out medium with sterilized cork borer (6mm) in each plate which was streaked with test bacterial. Uniform volume of different concentration 500 µg/ml and 250 µg/ml of the test synthesized compounds were placed in each of the cavity, after overnight incubation at37 °C. The diameter of inhibition zone formed around the well was measured in mm. DMSO was used as a solvent for all the compounds, which did not show any inhibition against test bacteria.

Table- 1: Physicaland Analytical data of the synthesized compounds (2-12)

Comp. No.	Molecular Formula	M.P °C	Color	Recrystal-lized Solvent	Yield (%)	Calc. (Found) %		
						C	H	N
2	$C_8H_5N_3S$	150	Off-white	Ethanol	51	54.84(54.79)	2.88(2.82)	23.98(24.05)
3	$C_9H_7N_3S$	oily	Red	Petroleum ether-Ethyl acetate	61	-----	-----	-----
4	$C_8H_7N_3OS$	186-188	Green	dioxin	59	50.25(50.19)	2.64(2.60)	21.98(22.02)
5	$C_8H_5N_3S_2$	246	Pale yellow	Ethanol	68	46.36(46.27)	2.43(2.39)	20.27(20.33)
6	$C_8H_5N_3OS$	>250 dec.	Purple	Ethanol	68	50.25(50.20)	2.64(2.60)	21.98(22.05)
7	$C_{15}H_{11}N_3OS$	122	Red	Ethanol	60	64.04(63.98)	3.94(3.89)	14.94(14.98)
8	$C_{15}H_{11}N_3S$	185	Pale-Yellow	Ethanol	34	67.90(67.85)	4.18(4.12)	15.84(15.90)
9	$C_{14}H_9N_3OS$	167	Pale-Green	Ethanol	55	62.91(62.85)	3.39(3.35)	15.72(15.77)
10	$C_{16}H_{14}N_4S$	190	Dark-Yellow	Ethanol	39	65.28(65.22)	4.79(4.75)	19.03(19.09)
11	$C_{14}H_8N_4O_2S$	177-179	Dark-Orange	Ethanol	47	56.75(56.69)	2.72(2.69)	18.91(18.95)

Results and Discussion:

For the synthesis of the 3-substituted Benzo [4,5] thiazolo [2,3-c] [1,2,4] triazole heterocyclic derivatives, the reaction sequences are outlined in scheme (1). The synthesis of 2-hydrazino-1,3-benzothiazole was the first step in the synthesis of the target compound. benzo[d]thiazole-2-thiol was treated with hydrazine hydrate to replace the thiol group by hydrazine group. Synthesis of new series of tricyclic compounds of 2-hydrazino-1, 3-benzothiazole are a new derivatives. The spectral data of compound (1)) showed the appearance of multiple bands in FTIR spectra at 3319.60 and 3201.94 cm^{-1}, 3355.50 cm^{-1} due to stretching vibrations of hydrazine $NHNH_2$ group bands and disapprarance of stretching band at 2585.29 for thiol group[5,6].

Synthesis of the target compoundes was achieved by cyclocondensation reaction of the hydrazine derivative (1) with triethyl-formate, acetic anhydride ,ethylchloroformate the corresponding fused binary systems , [1,2,4] triazoloben - zothiazole (2,3), triazolobenzothiazol-3(2H) - one (4) ,derivatives respectively (Scheme 1).

The IR spectrum of compound (4), showed a moderately strong band at 3344.79 cm^{-1} due to (NH) stretching vibrations, at 1678.13 cm^{-1} for (C=O of amide) and 1626.54 cm^{-1} for (C=N) stretching vibration .The IR spectrum of compound (4) showed the disappearance of he characteristic bands of ($NHNH_2$) group.

Further compound (5) was obtained in good yield when the hydrazide (1) was allowed to condense with carbon disulphide, in potassium hydroxide solution. The characteristic bands in FTIR spectrum of compound (5) showed bands 3353.54 cm^{-1} attributed to the (NH) group, 2890.89cm^{-1} refer to (SH) in tutomar and band at 1575.89 due to (C=N) stretching vibration , the important strong band at 1130.32 cm^{-1} due to (C=S) stretching vibration.

Moreover Reaction of the hydrazide (1) reacts with urea for synthesis of compound (6), The IR spectrum of this compound show band at 3319.60 cm^{-1} broad for (O-H) which give a good indication for the cyclization [12] , 1647.28 cm^{-1} (C=N), 1450.52 cm^{-1} (C-N), 1012.66 cm^{-1} for(C-O) stretching vibration.

The cyclization of the synthesized Schiff's bases (7-11), to produce the triazole ring, was done by the treatment of those bases with a solution of bromine in glacial acetic acid. The FTIR spectrum showed the disappearance of absorption band of (NH) group in Schiff's and appearance of a band at (1650.08-1666.39 cm^{-1}) due to (C=N) stretching vibration.

Finally the product (12) after isolation showed an IR spectrum which exhibited bands at 3371.68 and 3263.66 cm^{-1} (NH_2), band at 1647.26 cm^{-1} stretching vibration for(C=N),band at 1317.43 cm-1 due to (C-N) stretching vibration.

Antibacterial Activity:

All the synthesized compounds (2-12) were tested for antibacterial activity aganist bacterial species *Klebsiella sp., E. coli. Enterococcus sp., Lactobacillus sp.* DMSO was used as diluents to get desired concentration (500µg /ml and 250µg /ml) of drugs to test upon standard bacterial species.

The zone inhibition was measured in mm. The standard drug used was streptomycin. The results of activity summarized in Table 3.

From the table 3 it's clear that antibacterial results were found uneven. The compounds (4, 5, 6, 9, 10 and 12) showed good activity against all bacterial species.

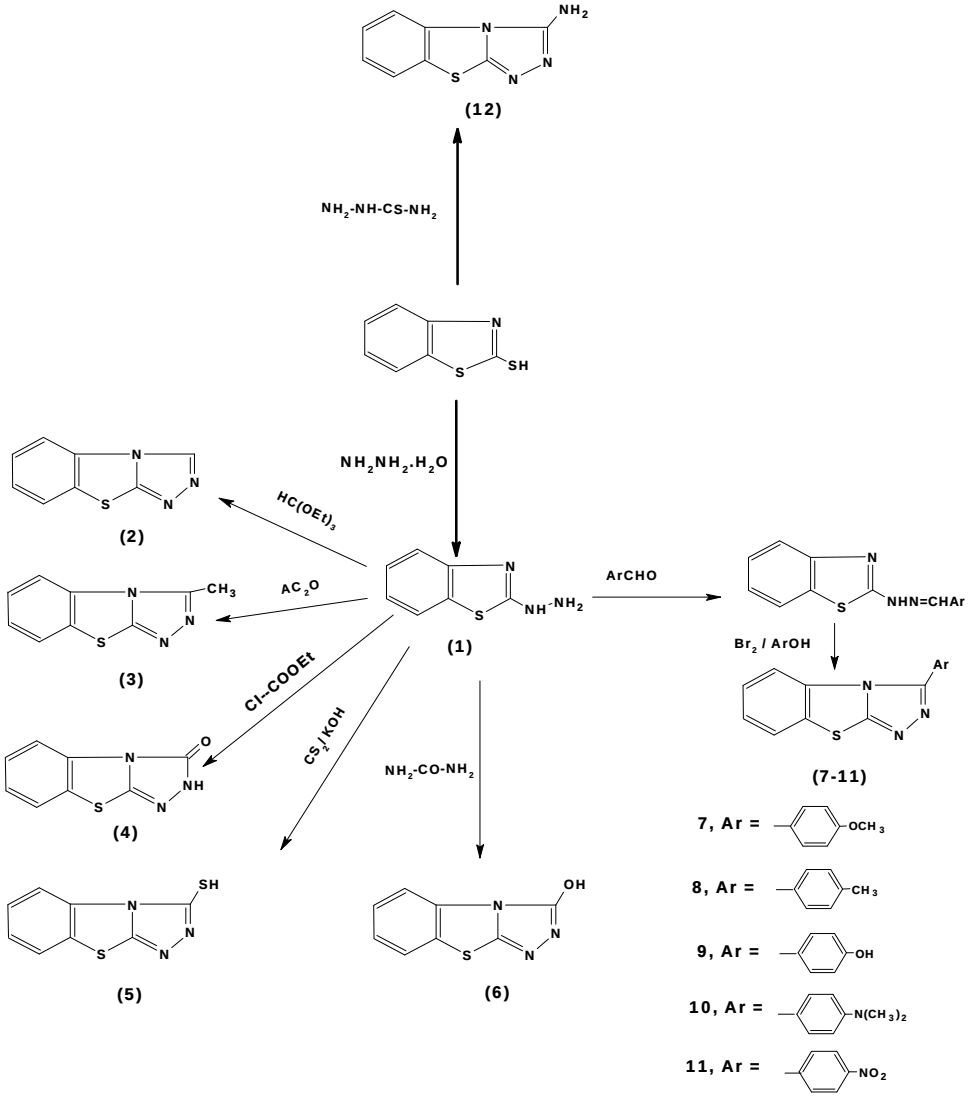
The compounds (2, 3, 7, 8, and 11) exhibited moderate antibacterial activity against *Klebsiella sp., E. coli. Enterococcus sp., Lactobacillus sp.*

The antibiotic stereptomycin shows 100% inhibition against all tested bacteria

Conclusions:

As for as stability studies of new hetrocycles is concerned it is reported that simple thiazole are stable to alkali but under drastic condition thiazole rings open up, much depends upon substituent present.

In present investigation we found triazolo benzothiazol ring is very stable to acid, alkali and heat treatment. Even ring isomerisation is not possible as was observed in triazolo benzothiazole. Sandwiching of thiazole ring between benzene and triazole prevents ring opening and imparts stability.



Scheme (1)

Table - 2: Spectral data for compounds (2-12)

Comp. No.	ν(C=C)	ν(C=N)	ν (C-S-C)	ν(C-H)al. ν(C-H)ar.	Others
2	1580.10 1548.50	1611.98	1192.45 1078.00 669.62	2900.07 3059.14	1060.88(C-N)$_{st}$
3	1586.17 1558.50	1626.54	1192.45 1078.00 669.62	2900.07 3059.14	1481.44 (C-CH$_3$)$_{st}$
4	1597.33 1488.00	1626.54	1192.45 1078.00 669.62	2900.07 3059.14	3344.79 (NH) $_{st}$ 1678.13(C=O) $_{st}$
5	1533.46 1549.10	1579.75	1192.45 1078.00 669.62	2897.20 3051.49	3353.54 (NH)$_{st}$ 1130.32 (C=S)$_{st}$ 2890.89 (SH)$_{st}$
6	1533.6 1618.33	1647.26	1192.45 1078.00 669.62	2901.21 3110.90	3319.60 (OH)$_{st}$ 1012.66 (C-O)$_{st}$ 1450.52(C-N)$_{st}$
7	1580.10 1548.50	1660.24	1192.45 1078.00 669.62	2897.20 3051.49	1390.89 (C-CH$_3$) $_{st}$
8	1580.10 1548.50	1649.06	1192.45 1078.00 669.62	2902.50 3070.19	3401.60 (OH)$_{st}$
9	1586.17 1432.31	1634.44	1192.45 1078.00 669.62	2902.50 3070.19	1537.22,1313.04 (NO$_2$)$_{st}$
10	1580.10 1548.50	1650.17	1192.45 1078.00 669.62	2902.50 3070.19	1230.76 (O-CH$_3$)$_{st}$
11	1586.17 1432.31	1590.78	1192.45 1078.00 669.62	2902.50 3070.19	1437.66 (N-CH$_3$)$_{st}$
12	1597.11 1558.54	1653.05	1192.45 1078.00 669.62	2872.10 3128.64	3371.68 , 3263.66 (NH$_2$)$_{st}$ 1317.43(C-N)$_{st}$

Table-3: Antibacterial activity of synthesized compounds (2-12) (Zone of inhibition in mm).*500㎎/ml and 250㎎/ml.

Compound No.	*Klebsiella sp.*	*E.coli*	*Enterococcus sp.*	*Lactobacillus sp.*
2*	11	11	10	11
3*	11	12	11	11
4*	15	15	13	14
5*	17	17	15	14
6*	17	16	16	15
7*	11	12	12	12
8*	12	11	11	11
9*	17	16	16	17
10*	15	14	15	13
11*	13	12	12	11
12*	17	17	15	13
Streptomycine*	24	20	21	20

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