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Synthesis and characterization of benzothiazole and 1, 2, 3 Triazole based bisheterocycles for anti-oxidant activity

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Abstract
Heterocyclic chemistry includes several chemical classes, azole being one of them. The study synthesis and characterization of benzothiazole and 1,2,3-triazole based bisheterocycles for anti-oxidant activity. we synthesize and assess the antioxidant activity of a library of bisheterocycles produced from 2-mercaptobenzothiazole. These bisheterocycles include benzothiazole and 1,2,3-triazole moieties linked by a Sulphur bond. This article provides the first comprehensive account of the synthesis and biological assessment of new bisheterocycles 1,2,3-triazoles. The synthesised compounds exhibited moderate antioxidant capabilities, with EC50 values above 76.7 µg/mL.

Keywords: Antioxidant, Benzothiazole and 1,2,3-triazole based bisheterocycles

1. Introduction

Heterocyclic chemistry has been at the forefront of the search for chemicals that improve human health and longevity from the field of organic chemistry's earliest days. Seventy percent or more of all pharmaceuticals are heterocyclic molecules. They play important roles in several biological processes and may be found almost everywhere. Isolated heterocyclic compounds from nature are often used as starting points for creating novel molecules with biological significance. The majority of heterocyclic medicines are also synthesized from commercially available fine chemicals. Synthesis and characterization of novel molecular entities containing heterocyclic structures are of paramount importance in this context. There are several reasons why this line of inquiry in organic chemistry should be pursued. First, it will aid in elucidating the unexplained inherent chemical behavior of tiny molecules. Second, it will be a huge boon to research into novel synthesis techniques. Thirdly, standards for characterizing related molecules might be established by characterizing a collection of compounds using spectrum approaches. Finally, synthesised compounds may be evaluated biologically to find potential lead compounds for structural fine-tuning. When it comes to the relevance of organic compounds, those that include one or more sulfur or nitrogen atoms are at the top of the list. [1]

2. Materials and methods

Although the demand for new chemical materials and biologically active molecules continues to grow, chemists have hardly begun to discover the enormous pool of potentially active compounds. In the scenario of a persistent request especially from pharmaceuticals companies for better drugs, it has become a challenging task for medicinal chemists to prepare new patentable molecules that combine high activity and selectivity, drug-likeness, and good pharmacokinetic properties.
As part of our continuing interest in the synthesis of biologically active compounds, we have successfully synthesized such derivatives which consist of two distinct pharmacophores; benzothiazoles and trizoles, each certainly, possessing a wide range of biological and pharmacological activities.

Benzothiazole scaffold derivatives consist of fused bicyclic ring systems. Benzothiazoles are an important class of potential organic molecules in medicinal chemistry due to their extensive range of activity such as neuron protective, anti-convulsive, anti-glutamate, anti-malarial, anthelmintic, anti-tubercular, analgesic, anti-inflammatory, anti-microbial, and anti-cancer to name a few. In this context, synthetically accessible molecules having new benzothiazole scaffolds with promising biological profiles have attracted the attention of medicinal organic chemists for their applications in potential chemotherapeutics.

2.1. Experimental Procedure:

Melting points were determined using Buchi-510 instrument. IR spectra were recorded on Perkin-Elmer-683 series spectrometer with KBr optics, and 1H NMR (300 MHz) were recorded on BrukerAvance 400 spectrometer using TMS as internal standard (chemical shifts and ppm). Mass spectra were recorded on a VG micromass70-70 H instrument. CHN analysis was carried out using Vario Micro Cube Elementar instrument.

2.2 General method of synthesis for 1,2,3- benzothiazole and 1,2,3-triazole

2.2.1. 4-Methyl-3-Nitrobenzoic Acid (2)

HNO_3 (10.05 g, 125.64 mmol, 1 equiv.) was added to a stirred solution of 4- methylbenzoic acid (18 g, 132.35 mmol, 1 equiv.) in Dichloromethane and stirred for 10-15 minutes. Then conc. H2SO4 (24.46 ml, 249.66 mmol,) was added to the above reaction mixture drop wise at 0 ℃ for a period of 15-20 minutes with vigorous stirring. Stirring was continued at room temperature for a period of 4-5 hours till TLC showed the completion of the reaction. The reaction mixture was quenched with ice cold water (200 mL) and then allowed to return rt. The organic layer was separated and evaporated in vacuo under reduced pressure. The resulting residue was washed with water several times to remove acidic impurities. It was filtered off to give crude solid which on Recrystallization using EtOAc.

2.2.2. N-(4-Methoxyphenyl)-4-Methyl-3-Nitrobenzamide (3)

Compound **2** (20 g, 110.49 mmol, 1 equiv.) was converted to its acid chloride (21.10 g, 110.45 mmol, 1 equiv.) using SOCl2 (12.02 g, 101.00 mmol, 1.5 equiv.) and dry benzene at 80 ℃ in the presence of catalytic amount of DMF (2-3 drops) in 96 % yield. This freshly prepared acid chloride was added drop wise to stirred solution of *p*-anisidine (13.04 g, 106.01 mmol, 1 equiv.) and Et3N (16.09 g, 159.00 mmol, 1.5 equiv.) in dry THF (35 mL) at 0 ℃ and the stirring was continued at room temperature for a period of 2-3 hours. Solvent THF was removed by rotaevaporator under reduced pressure. The crude solid was washed with a saturated solution of NaHCO3, 1N HC1 and cold water to remove if any unreacted starting materials were present. The crude solid was filtered off through Buchner funnel and crystallized using methanol to obtain light yellow crystals of **3.**

2.2.3. N-(4-Methoxyphenyl)-4-Methyl-3-Nitrobenzothioamide (4)

To a stirred solution of amide **3** (21 g, 73.42 mmol, 1 equiv.) in dry toluene (50 mL), Lawesson's reagent (14.75 g, 36.71 mmol, 0.5 equiv.) was added at 90 ℃. The reaction mixture was refluxed for 2-3 hrs. After completion of the reaction (monitored by TLC) solvent toluene was removed by vacuo under reduced pressure. The resulting reaction mixture was quenched with 10 mL of Sodium hypochlorite aqueous solution and ice-cubes were added to it. Then the reaction mixture was filtered through Buchner funnel to get dark yellow coloured crude product. Purification of the crude solid by column chromatography on silica gel using EtOAc: petroleum ether (2:5) afforded pure pale yellow coloured compound **4.**

2.2.4. 6-Methoxy-2-(4-Methyl-3-Nitrophenyl)- 1, 3-Benzothiazole (5)

Dess-Martin periodinane (25.27 g, 59.59 mmol, 1.2 equiv.) was added to a stirred solution of thioformanilide **4** (15.00 g, 49.66 mmol, 1 equiv.) in dichloromethane (100 mL) at room temperature. The progress of the reaction was monitored by TLC. After the completion, the reaction mixture was quenched with H2O (2 x 10 mL) and it was extracted with CH2Cl2 (3 x 10 mL). All organic layers were combined and dried over anhydrous Na2SO4 and the solvent was removed in vacuo, to afford the crude product. Then it was purified by column chromatography on silica gel using EtOAc: petroleum ether (1:3) to get the 2-aryl benzothiazole **5** as light yellow colored solid.

2.2.5. 4-(6-Methoxy-1, 3-Benzothiazole-2-yl)-2-Nitrobenzoic Acid (6)

Freshly prepared Tetrabutylammonium Permanganate (TBAP) (25.34 g, 70.00 mmol, 2.1 equiv.) was added to a solution of 2-arylbenzothiazole **5** (10 g, 33.33 mmol, 1.0 equiv.) in dry pyridine (50 mL) at room temperature. It was observed that the reaction was so exothermic, the reaction mixture started to reflux for 5-10 minutes even at room temperature. The reaction was continued to stir at room temperature for a period of 12 hours. The completion of reaction was monitored by TLC. This reaction mixture was poured into a mixture of NaHCO3 and cold dilutes Hcl. Then, the reaction mixture was extracted with ethyl acetate (3x10 mL). The combined organic layer was removed by vacuo under reduced pressure to afford crude compound. Recrystallization using EtOAc: petroleum ether resulted into a free flowing light yellow colored solid **6.**

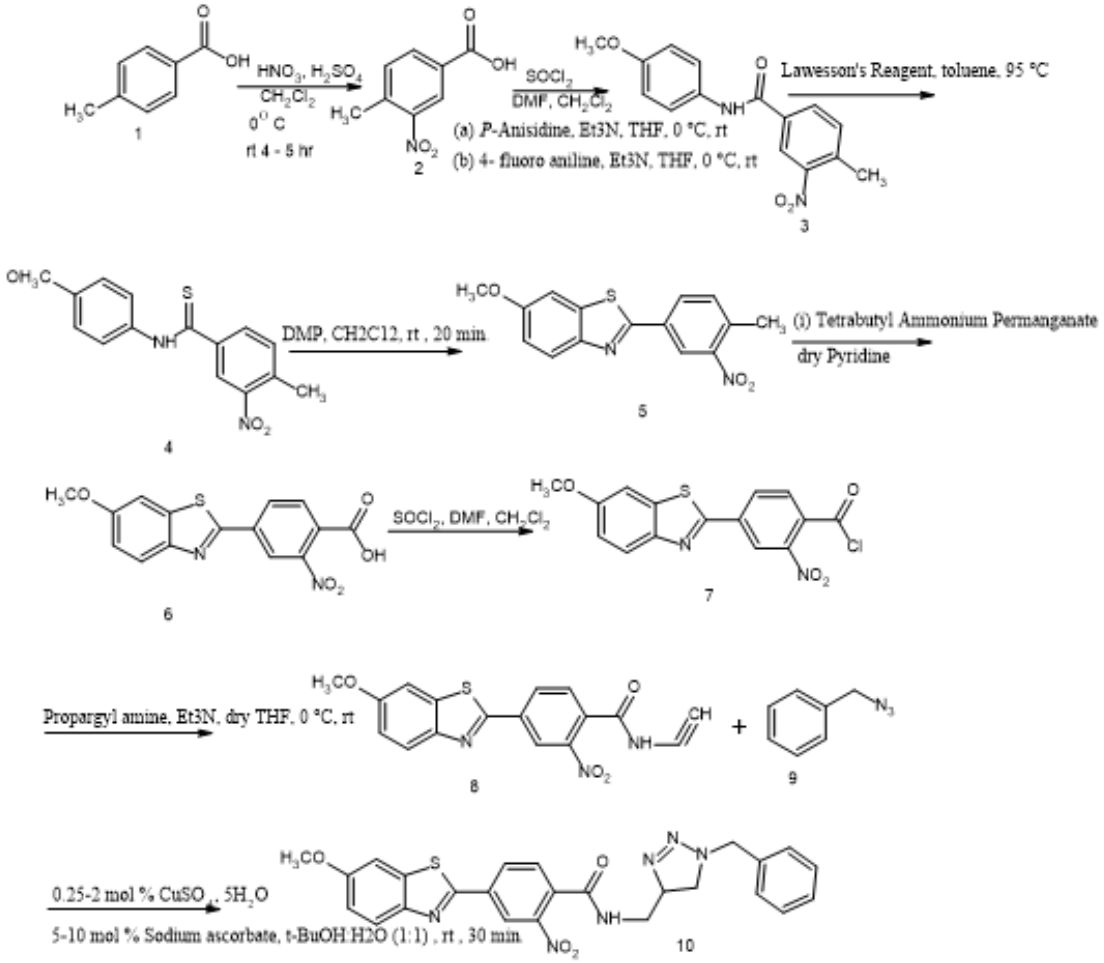
2.2.6. 4-(6-Methoxybenzo [*d*] Thiazol-2-yl)-2-Nitro-*N*-(Prop-2-ynyl) Benzamide (7-8)

Nitro acid **6** (5.0 g, 15.15 mmol, 1.0 equiv.) was converted to its acid chloride **7** (5.11 g, 14.68 mmol, 1.0 equiv.) in the presence of SOCl2 (1.64 mL, 13.78 mmol, 1.5 equiv.) and catalytic amount of DMF (2-3 drops) in dry benzene in 97 %. This freshly prepared acid chloride was added drop wise to stirred solution of Propargyl amine (0.96 g, 17.45 mmol, 1.2 equiv.) and Et3N (2.64 g, 26.08 mmol, 1.5 equiv.) in dry THF at 0 ℃. The stirring was continued further at room temperature for a period of 3- 4 hours. Solvent THF was removed in vacuo under reduced pressure. The resulting crude solid was extracted with ethyl acetate (3 x 50 mL), washed with a saturated solution of NaHCO3, 1N Hc1 and cold water to remove if any unreacted starting materials were present. The combined organic layers were distilled by vacuo to afford solid compound which was recrystallized from methanol to obtain yellow crystals of **8.**

2.2.7. Synthesis of *N*-((1-benzyl-l*H*-l, 2, 3-triazol-5-yl) methyl)-4-(6-methoxybenzo[*d*] thiazol-2-yl)-2-nitrobenzamide (10)

Water and tertiary alcohol in the ratio (1:1) were added to the

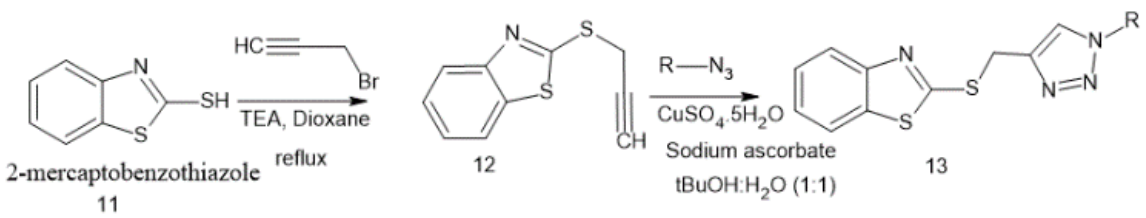
round bottom flask containing compounds **8** possessing triple bond and freshly prepared benzyl azide **9a** and stirred for 5-10 minutes. To this reaction mixture were added 0.5 mol % CuSO4.5H2O and 10 mole % Sodium ascorbate simultaneously. Reaction was continued for 12 hours till the completion of the reaction (confirmed with TLC). After the completion of the reaction, the reaction mixture was worked up with ethyl acetate, washed with brine and dried over Na2SO4. The organic layer was separated and removed in vacuo under reduced pressure. The resulting material was purified by column chromatography by using ethyl acetate and hexanes (8:2) to afford colorless compound 10.



Scheme 1

2.3 General procedure for synthesis of 1, 2, 3-triazole derivatives

2-mercaptobenzothiazole (11) was used as starting material and was refluxed with propargyl bromide in dry THF. Various substituted aromatic/sugar azides were reacted with propargylated 2-mercaptobenzothiazole (12) under click chemistry reaction conditions to obtain the novel *bis*-heterocycles (13) in quantitative yields. Compound 12 was dissolved in 20 mL of *t*-Butanol: water (1:1) solvent at ambient temperature. CuSO4.H2O was then charged and the reaction mixture was stirred for 5 min. Reaction mixture was light blue in colour. Sodium ascorbate was now added at once to the reaction mixture and stirred for 15 min. Reaction mixture colour was changed to dark yellow. After 15 min., azide was added. The reaction mixture was allowed to stir for further 8 h at ambient temperature. After the completion of the reaction monitored by TLC, reaction mixture was quenched with water and extracted with ethyl acetate. Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to obtain the required product.



Scheme - 2

2.4. In vitro antioxidant studies

Antioxidants are intimately involved in the prevention of cellular damage: the common pathway for cancer, aging, and a variety of diseases. A free radical may be an atom or molecule with one or more unpaired electrons. The free radicals are capable of dependence existence and cause oxidative tissue damage. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.

2.4.1. DPPH radical scavenging assay [2-5]

The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The antioxidant reacts with stable free radical, DPPH (2,2-diphenyl-1-picrylhydrazyl) and converts it to 1,1-diphenyl-2-picrylhydrazine. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, it is decolorized which can be quantitatively measured from the changes in absorbance.

100 µL of various concentrations of test compounds were added to respective wells of a 96-well micro plate. Equal amount of DPPH was also added to each well to make up a final volume of 200 µL. After 20 min incubation in the dark, the ability of test compounds to scavenge the free radical DPPH was measured by recording the absorbance at 517 nm using an ELISA plate recorder. Experiment was performed in triplicates and average values were considered. An equal amount of methanol and DPPH was added to the control. Ascorbic acid was used as reference standard.

$$\%\ scavenging\ of\ DPPH = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2.4.2 ABTS radical scavenging assay [2-5]

ABTS is chemically 2,2-azinobis-3-ethylbenzo-thiazoline-6-sulphonic acid. The reduction of this radical by test compounds was measured at 690 nm. The electron transfer capability of test compounds was studied using ABTS radical scavenging assay. In a 96-well microtitre plate, 40 µL of the test compound / Ascorbic acid, 200 µL of methanol and 30 µL of ABTS solution were added. The plate was then incubated at 37°C for 20 min after which the absorbance was measured at 690 nm using an ELISA plate reader.

Sample blank and control were also taken. The experiment was performed in triplicates and average values were considered.

$$\%\ scavenging\ of\ ABTS = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

3. Result and Discussion

3.1 Physical characterization

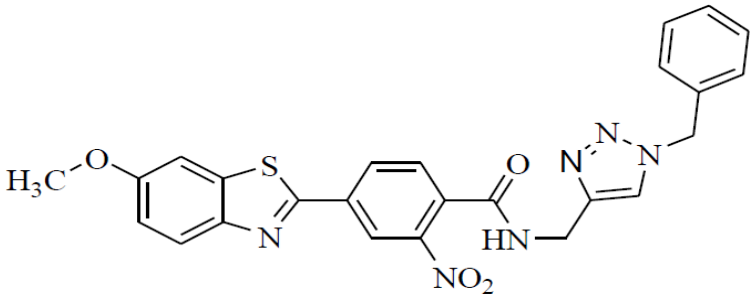
Table 1: Physical Characterization Data of Compounds

Sl. No.	Compound code	Molecular formula	Molecular weight	% of Yield	Melting Point (°C)
Scheme 1					
1	**10a**	$C_{25}H_{20}N_6O_4S$	501	87	205.4-206.6
2	**10b**	$C_{22}H_{20}N_6O_6S$	497	**85**	196-197
Scheme 2					
3	**13**	C16H11BrN4S2.	403	97	148-150

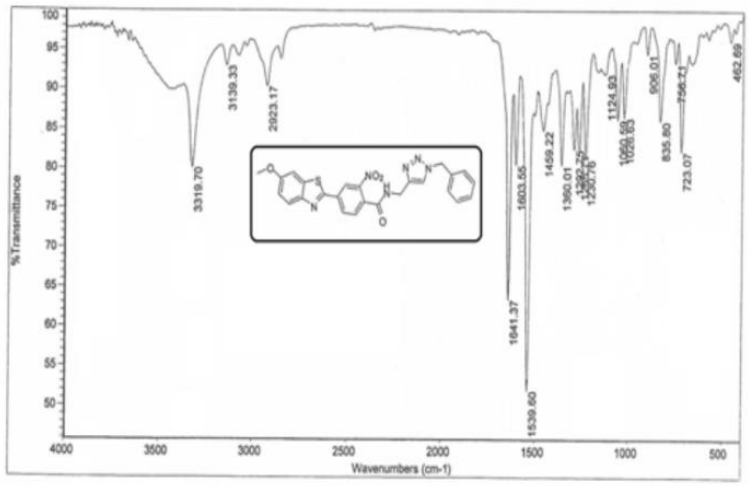
3.2 Spectral analysis

N-((1-benzyl-1*H*-l, 2, 3-triazol-5-yl) methyl)-4-(6-methoxybenzo[*d*]thiazol-2-yl)-2- nitrobenzamide (10a)

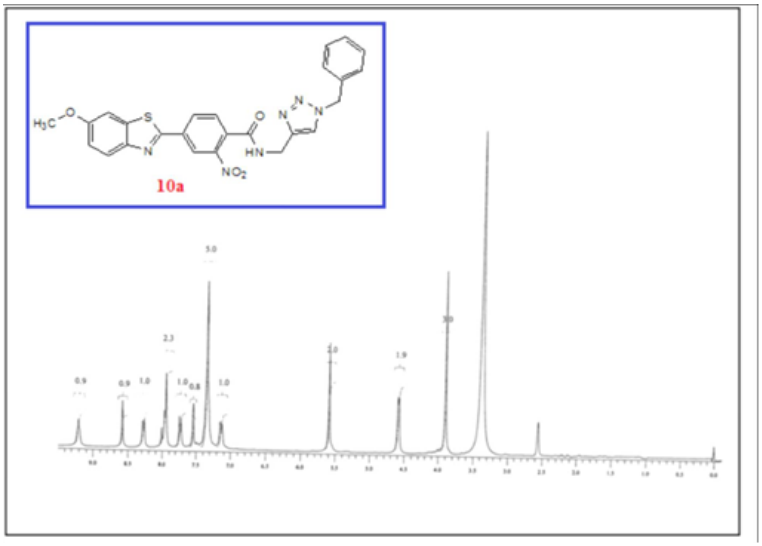
Molecular structure



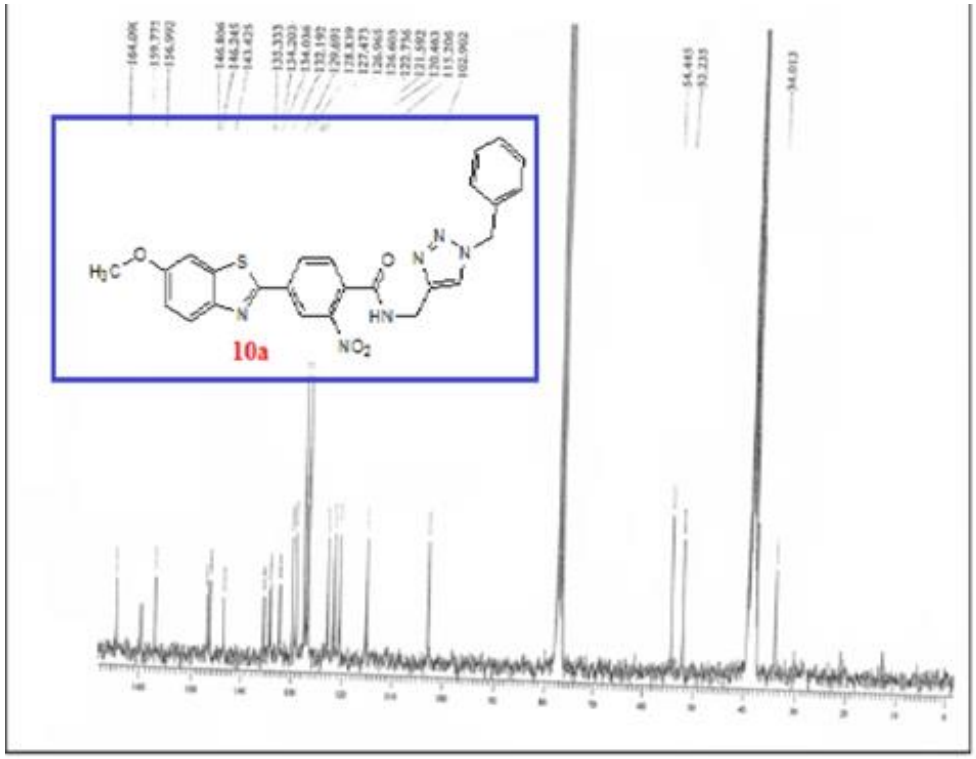
Yield (%)	: 87
M.P (ºC)	: 205.4-206.6
I.R (Neat-cm^{-1})	: 1539 (-NO2), 1649(-CONH2).
^{1}H NMR (DMSO-*d6* -300 MHz)	: δ 3.91 (s, 3H, OCH3), 4.51-4.65 (d, 2H, *N*-CH2), 5.58 (s, 2H, CH2), 7.05-7.20 (d, 1H, Ar-H), 7.25- 7.43 (s, 5H, Ar-H), 7.54 (s, 1H, Ar-H), 7.65-7.80 (d, 1H, J= 7.93 Hz), 7.84-8.05 (m, 2H, Ar-H), 8.21-8.36 (d, 1H, J = 7.74 Hz Ar-H), 8.58 (s, 1H, Ar-H), 9.20 (brs, 1H, NH).
^{13}C NMR (DMSO-*d6* -75 MHz)	: δ 34.01, 52.23, 54.44, 102.90, 115.20, 120.46, 121.59, 122.73, 126.60, 126.96, 127.47,128.83, 129.69, 132.19, 134.03, 134.20, 135.33,143.42, 146.24, 146.80, 150.99, 159.77, 164.09.
Mass (ESI)	: 501 (M++H).
CHN-Analysis	: Anal. Calcd. For $C_{25}H_{20}N_6O_4S$: C, 59.99; H, 4.03; N, 16.79; S, 6.41; found: C, 59.90; H, 4.07; N, 16.83;S, 6.42%.



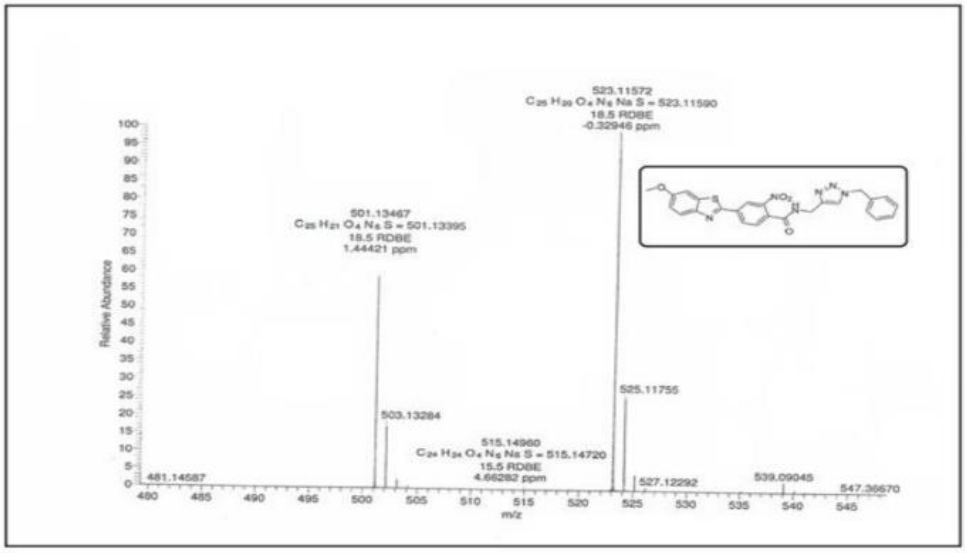
IR Spectrum of Compound 10a



Compound 10a: ^{1}H NMR



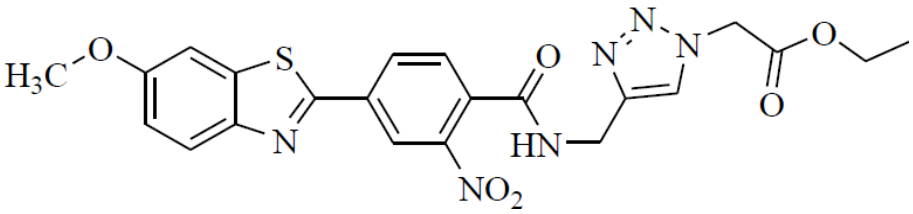
13C NMR Spectrum of Compound 10a



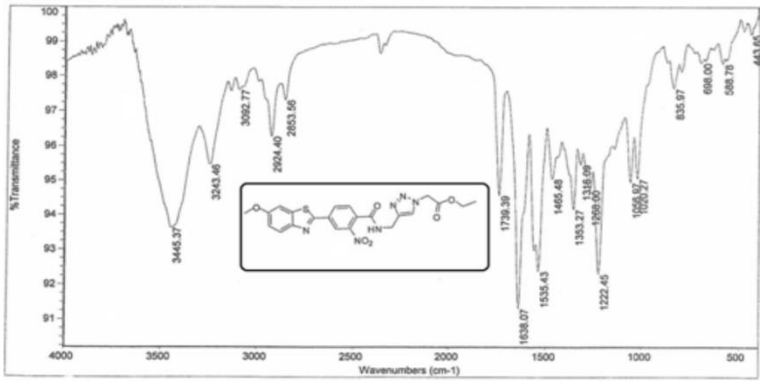
ESI-MS spectrum of Compound 10a

Ethyl-2-(5-((4-(6-methoxybenzo[*d*]thiazol-2-yl)-2-nitrobenzamido) methyl)-1*H*-l, 2, 3-triazol-1-yl) acetate (10b)

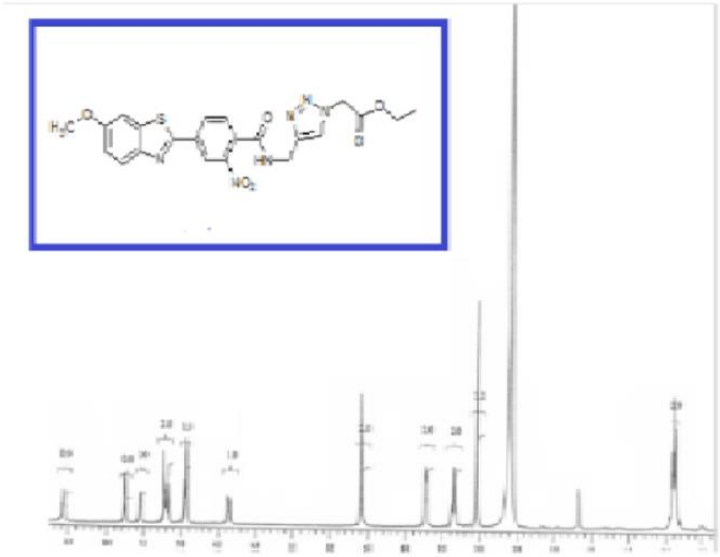
Structure



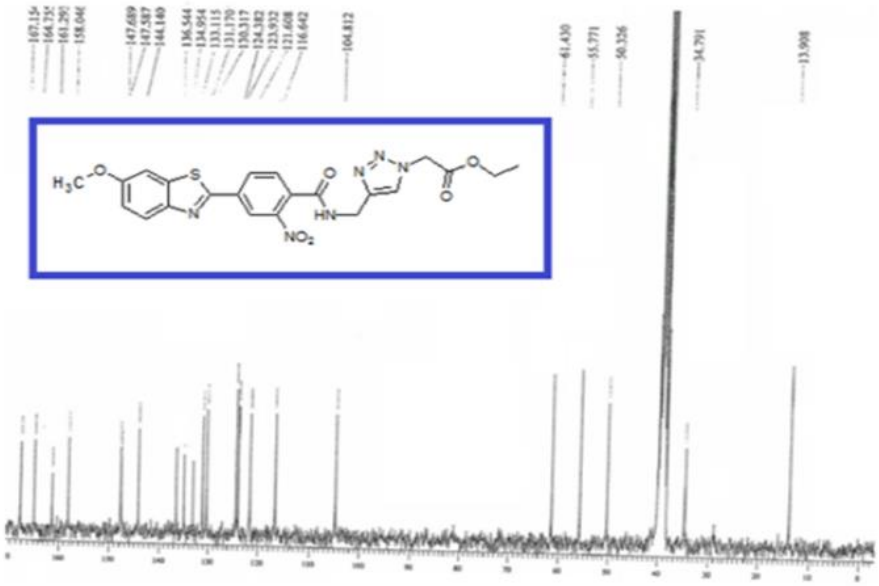
Yield (%)	: 85
M.P (ºC)	: 196-197
I.R (Neat-cm^{-1})	: 1535 (-NO2), 1638(-CONH).
^{1}H NMR (DMSO-*d6* -300 MHz)	: δ 1.23 (t, 3H, CH3), 3.87 (s, 3H, CH3), 4.09-4.27 (q, 2H, O-CH2), 4.47-4.65 (d, 2H, *N*-CH2), 5.40 (s, 2H, CH2), 7.06-7.27 (m, 1H, Ar-H), 7.69-7.83 (m, 2H, Ar-H), 7.93-8.14 (m, 2H, Ar-H), 8.28- 8.43 (m, 1H, Ar-H), 8.58 (s, 1H, Ar-H), 9.38 (brs, 1H, NH).
^{13}C NMR (DMSO-*d6* -75 MHz)	: δ 13.90, 34.79, 50.32, 55.77, 61.43, 108.41, 116.64, 121.60, 123.93, 124.38, 130.31, 131.17, 133.11, 134.95, 136.54, 144.14, 147.58, 147.68, 158.04, 161.29, 164.75, 167.15.
Mass (ESI)	: 497 (M++H).
CHN-Analysis	: Anal. Calcd. For $C_{22}H_{20}N_6O_6S$: C, 53.22; H, 4.06; N, 16.93; S, 6.46, found: C, 53.12; H, 4.11; N, 16.96; S, 6.48 %.



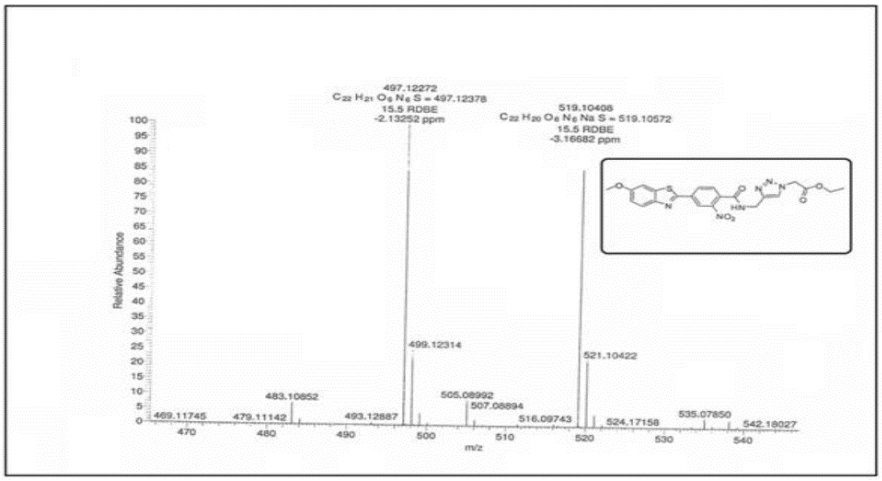
IR spectrum of compound 10b



1H NMR spectrum of compound 10b



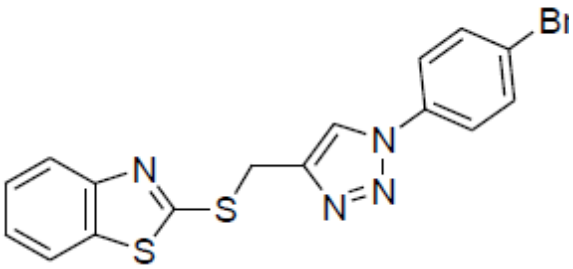
13C NMR spectrum of compound 10b



ESI-MS spectrum of compound 10b

2-[(1-(4-bromophenyl)-1H-1, 2, 3-triazol-4-yl) methylthio]benzo[d]thiazole (13)

Structure



Yield : 97%

m.p. : 148-150 °C

IR (KBr) (cm^{-1}) : 3126, 3058, 2394, 1496, 1458, 1426, 1050, 999, 821, 746, 722.

^{1}H NMR (CDCl3, 200 MHz): δ 4.77 (s, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J*= 7.1 Hz, 1H), 7.55-7.61 (m, 4H), 7.77 (d, *J* =7.4 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 8.06 (s,1H).

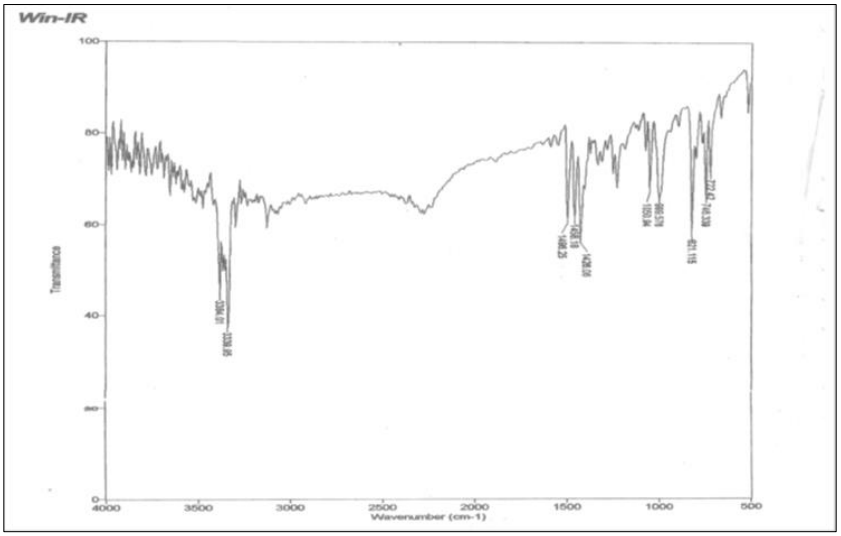
^{13}C NMR (CDCl3, 75 MHz): δ 27.56, 121.20, 121.49, 121.96, 122.48, 124.52, 126.19, 132.88, 135.54, 135.90, 152.98, 165.69.

Maldi-MS : 403 (M+), 405 (M++2).

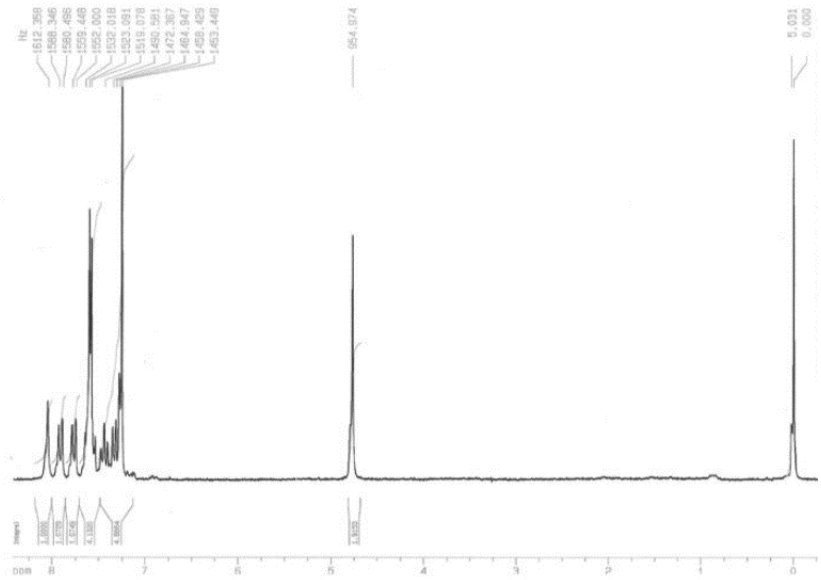
Elemental Analysis : Calculated for molecular formula C16H11BrN4S2.

Calculated: C, 47.65; H, 2.75 N, 13.89.

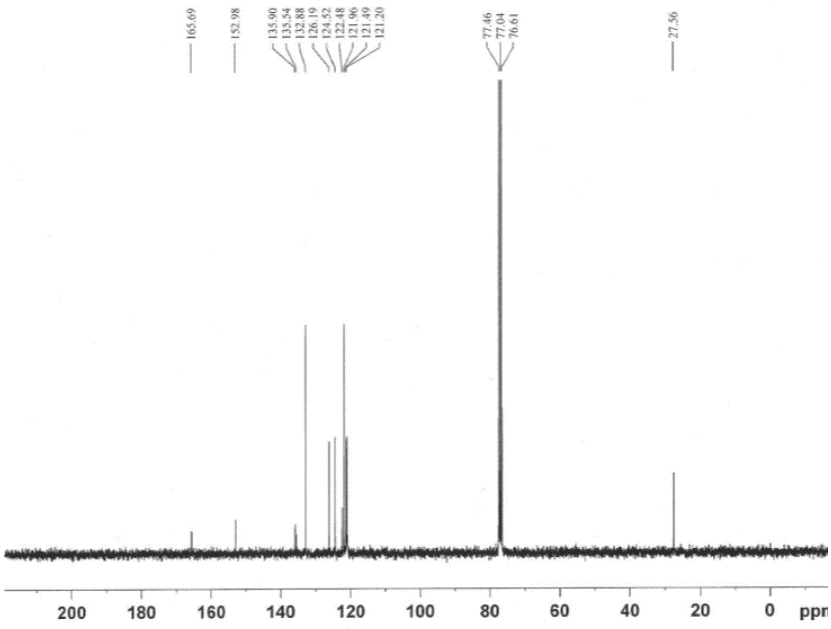
Found: C, 47.57; H, 2.69; N, 13.91%.



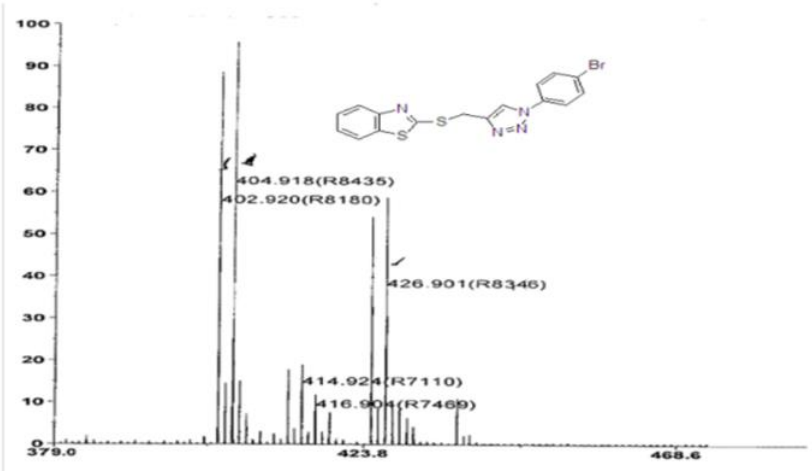
IR spectrum of compound 11



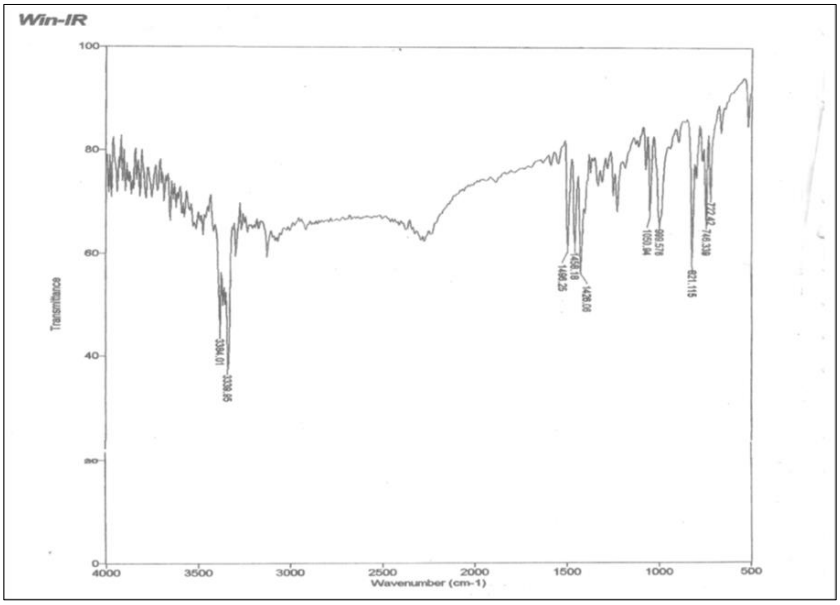
^{1}H NMR spectrum of compound 13



13 CNMR spectrum of compound 13



Mass spectrum of compound 13



IR spectrum of compound 13

4.4 In vitro antioxidant activity

The evaluation of the antioxidant activity of compounds was carried out through two assays based on the detection of DPPH and ABTS radicals. These assays are widely used because of their simplicity, speed, and sensitivity. Antioxidant activity was expressed as the effective concentration of the sample that led to a 50% reduction (EC50) in the initial concentration of DPPH (or ABTS). The EC50 values obtained for compounds are listed in Table 2.

It was observed that compounds had EC50 values ranging from 76.7 to 136.7 µg/mL in the DPPH assay and from 113.2 to 442.6 µg/mL in the ABTS assay, indicating generally low antioxidant activities of 5a-e when compared to those of standards (ascorbic acid and TROLOX).

Compound 13 was more active than other two compounds.

Table 2: Antioxidant activity of synthesized compounds

Compound	EC50 µg/mL	
	DPPH	ABTS
10a	102.5±6.4	165.2±9.0
10b	136.7±6.3	442.6±18.5
13	76.7±4.5	113.2±7.6
Ascorbic acid	1.6±0.2	
Trolox		4.1±0.4

5. Conclusion

In the present study, a focussed library of 2-mercaptobenzothiazole derived *bis*heterocycles encompassing benzothiazole and 1,2,3-triazole moieties conjugated through a sulphur linkage are synthesized and evaluated for their antioxidant activity. This work describes, for the first time, the synthesis and biological assessment of new *bis*heterocycles 1,2,3-triazoles. Moderate antioxidant potential was observed for synthesised compounds with EC50 values above 76.7 µg/mL.

6. References

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