Synthesis, Characterization and study of antibacterial activity in vitro of some hydrazones and formazan dyes containing benzothiazole moiety

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Abstract:

2-mercaptobenzothiazole (I) on treatment with hydrazine hydrate and HCl by two methods, µW and direct heating with solvent, give 2- hydrazinobenzothiazole (II), which on condensation by µW and direct heating with substituted aromatic aldehydes affords aryl substituted (1,3-benzothiazole-2-yl)hydrazone (III). The latter on treatment with aryl diazonium chloride at low temperature degree furnishes respective 1-(benzothiazol-2-yl)-5-(4-sub.phenyl)-3-arylformazan(IVa-e,Va-e,VIa-e)(formazan dyes) which on have been used as pigments for cotton, wool and white wood, and showed a significant value and also showed a resistance for primary washing process.

The antibacterial activities of these compounds have been assayed against various Gram -ve and Gram +ve organisms. The structures were elucidated and spectral behaviors were investigated with the use of ^{1}HNMR, ^{13}CNMR, FTIR, UV–Vis spectra .

تحضير وتشخيص ودراسة الفعالية المضادة للبكتريا في الاطباق لبعض الهيدرازونات واصباغ الفورمازان الحاوية على البنزوثايازول

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الكلمات المفتاحيه: 2-هيدرازينوبنزوثايازول,هيدرازون, فورمازان, الفعالية المضادة للبكتريا

الخلاصة:

من معاملة 2-مركبتوبنزوثايازول(I) مع الهيدرازين المائي وحامض الهيدروكلوريك وبطريقتي الاشعة الدقيقة او بالتسخين المباشر, مع الديهايدات اروماتيه معوضه للحصول على اريل معوض(3,1-بنزوثايازول-2-يل)هيدرازون (III), عومل الاخير مع اريل كلوريد ملح الدايازونيوم عند حراره منخفضه ليعطي على اصباغ الفورمازان 1-(بنزوثايازول-2-يل)-5-(4-معوض-فنيل(-3-اريل فورمازان (IVa-e,Va-e,VIa-e) الترتيب , والتي استخدمت كاصباغ للقطن والصوف ونشارة الخشب البيضاء والتي اظهرت مقاومة لعمليات الغسل الابتدائية.

الفعالية المضادة للبكتريا لهذه المركبات اختبرت ضد جراثيم سالبة وموجبة لصبغة كرام, وان التركيب تم تشخيصة بواسطة اطياف كل من ^{13}CNMR, FTIR ^{1}HNMR وكذلك UV–Vis .

Introduction

The formazans and their metal complexes have been used as dyes[1-4]. The chemistry of such dyes has long been of interest in the industry.

Formazans have been found to possess important medical applications; the formazans and heterocyclic hydrazones are known for their spectrum of biological activities such as antiviral [5,6] antimicrobial [7], anti-inflammatory [8], antifungal [9], anticancer [10], anti-HIV(Human immunodeficiency virus) [11,12]. Several formazans show promising antifertility [13] and anti-parkinsonian activity [14-17].

Furthermore the formazan nucleus is pharmacophoric in nature [18,19]. It was envisaged that the compounds containing these moieties in their molecular frame work might show enhanced biological activity.

Experimental :

Melting points were determined in open capillaries on Electrothermal melting point apparatus(Electrothermal Engineering LTD S-N 10853) and are uncorrected. ^{1}HNMR & ^{13}CNMR spectra were recorded on a Bruker AM 300 (300 MHz) instrument using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in parts per million (ppm). Splitting patterns are designated as follows: s- singlet, d- doublet, t-triplet, q- quartet and m- multiplet. The reactions were followed on pre-coated TLC plates (Silica gel 60 F254, Merck), visualizing the spots in ultraviolet light.The IR spectra were recorded on Shimadzu FTIR 8400S by using KBr disc method scan(400-4000)cm^{-1}. The UV measurements were obtained using Schimadzu (UV-Visible) spectrophotometer UV-1650 PC.

Antimicrobial Activity

All the newly synthesized compounds were screened for their antimicrobial activity against *Escherichia.coli, pseudomonas aeruginosa, staphylococcus aereus* and *Sal. typhi* using EtOH as solvent at 50 and 100µg/ml concentration by using cup-plate method. After 24hrs of incubation at 37 ^{o}C the zone of inhibition were measured in mm .

Synthesis of 2-hydrazino benzothiazole[20] (II)

Method 1: Concentrated HCl, (1.5ml) was added drop wise to hydrazine hydrate, (10ml, 80%) at 5-10ºC followed by ethylene glycol (40ml).To the above solution 2-mercaptobenzothiazole, (0.01mol, 1.67g) was added in portions. It was then refluxed for 3-4 hrs, cooled and poured onto crushed ice. The separated white solid was filtered, dried and recrystallized from ethanol, m.p.=198-200 , yield=90% .

Method 2: by µW: Dissolved (0.03 mol, 5 g) of 2-mercaptobenzothiazole in hydrazine hydrate (20ml,80%), in suitable flask and heat-resistant, the mixture under rays microwaves 30 minutes, cold mixture and condensed under vacuum at room temperature, precipitation is filterated and washed and recrystallized from ethanol, see Table-1.

Synthesis of aryl substituted (1,3,- benzothiazole-2-yl) hydrazones (IIIa-i) [21]

Method 1: 2-hydrazinobenzothiazole(II), (16.52g, 0.1mol) and appropriate substituted aldehydes (0.1mol) were refluxed in ethanol in presence of acetic acid on a water bath for 4hrs.The resultant solution were cooled and poured on to crushed ice. The solid that separated was filtered and recrystallized from ethanol or methanol.

Method 2: by µW with solvent free : Mixed (0.02 mol 3.304 g) of 2 - hydrazinobenzothiazole with (0.02 mol 2.02 g) of benzaldehyed or one its derivatives, in resistant heat beaker, the mixture under rays microwaves 5 - 10 minutes, stirring with a glass rod until the changing nature of the reactants in terms of color and nature, the resulting collection and recrystallized from ethanol , see Table-1.

General procedure for the prepare of formazans:

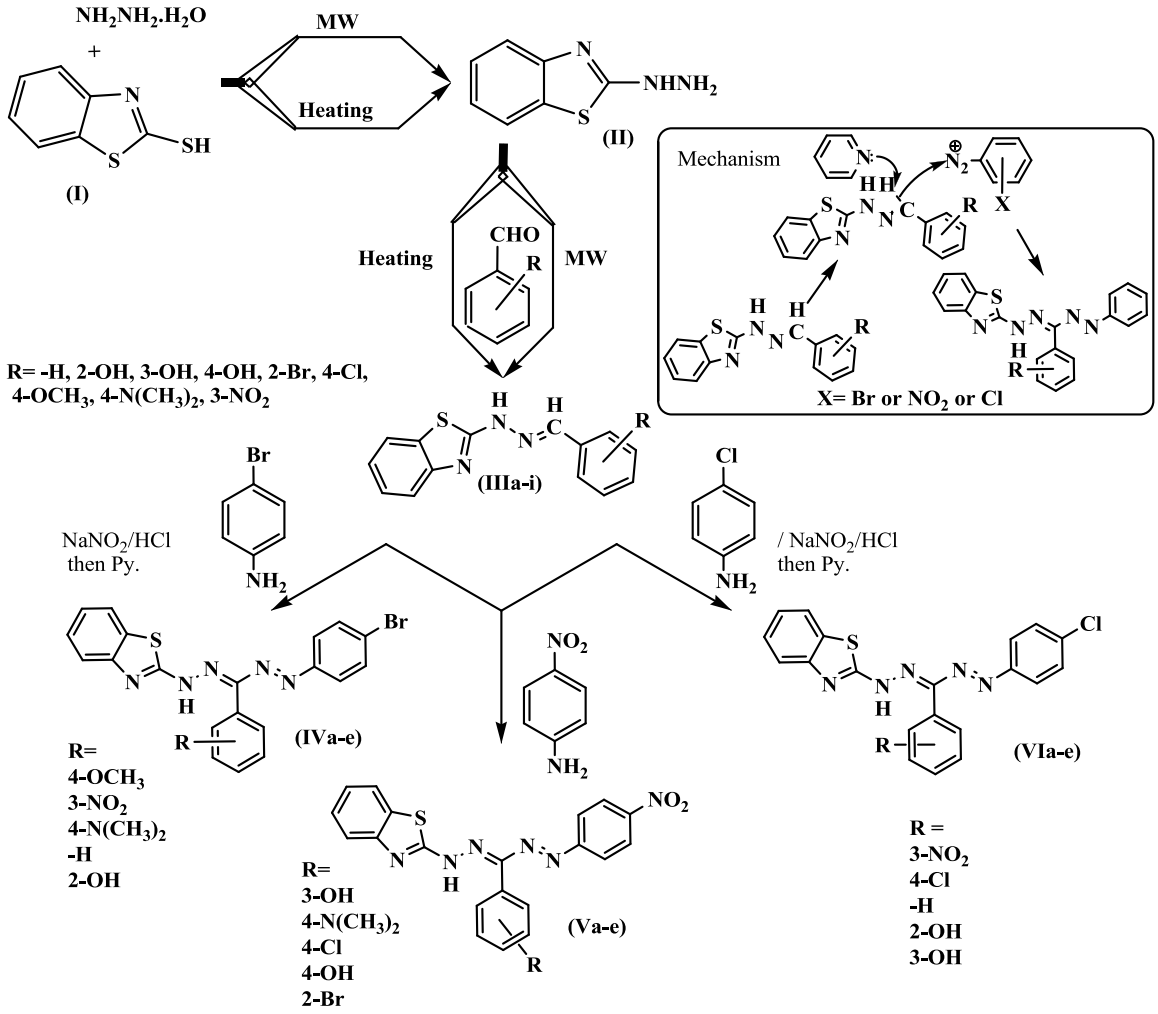
Synthesis of 1-(benzothiazol-2-yl)-5-(4-sub.phenyl)-3-arylformazan (IVa-e,V a-e,VI a-e)[22]

Aniline derivatives (0.02mol) in glacial acetic acid (2ml) and HCl (1.5ml) was diazotized with sodium nitrite (0.2g in 2ml water) at 0-50ºC. The resultant phenyldiazonium chloride solution was added with stirring to compound **3** (0.01mol) in pyridine (3ml) in ice-cold condition. The reaction mixture was left overnight at room temperature and poured into cold water (250ml) with continuous stirring when a dark coloured solid separated out. It was filtered, washed repeatedly with distilled water and recrystallized from methanol to afford the title compounds :

General procedure for a painting test : **dyeing[23]:**

Solved (0.1 g) of prepared formazan derivatives in 25 ml of ethanol in a beaker (100 ml), were taken equal pieces weight of both cotton and wool as well as white wood powder and put all of these types of materials in the flask dye solution inside complete with stirring for 30 minutes , left the flask in a hot air oven at 70° C limit drought, then washed test materials (cotton, wool, white wood powder) by three steps , first with water and the second with soap and third with liquid soap (open quantities) were observed proven color dye on each of them. see Table-2 .

Results and discussion



Scheme – 1: Reactions pathways for prepared compounds**.**

Organic part, The compounds (II-VI) were prepared according to Scheme-1, Condensation of 2-hydrazino benzothiazole (II) with substituted benzaldehyde in presence of glacial acetic acid in absolute ethanol as a reaction medium or by solventfree, fusion method gave schiff base (IIIa-i). products were confirmed by appearance of IR band in the region (1608-1627) cm^{-1} due to N=C group, (3190-3413) cm^{-1} due to NH group (secondary amine) and disappearance of IR band in the

region 3384 cm^{-1} and 1705 cm^{-1} corresponding to NH_2 group and CHO group of 2-hydrazino benzothiazole (II) and benzaldehydes respectively, see Table-3 .

Formation of formazan by diazo coupling reaction and products were confirmed by appearance of IR band in the region (1606-1635) and (1521-1528) cm^{-1} due to N=C and N=N groups, (3124-3448) cm^{-1} due to NH group (secondary amine), see Table-4 .

$^{1}H,^{13}CNMR$(DMSO d6) δ (ppm) Spectra showed the following data: (II) $^{1}HNMR$ 5.02 (s,2H,NH_2), 6.98 (t,2H,Ar-H), 7.19(t,2H,Ar-H), 7.34-7.49 (dd,2H, Ar-H), 8.99 (s,1H,NH). (II) $^{13}CNMR$: 133.69 (N=C-N thiazole), 131.64 (=C-S thiazole ring),128.60 (=C-N in thiazole ring) , 127.26, 126.50 (2CH Ar near thiazole ring), 122.35, 122.06 (CH far thiazole ring). (IIIa)$^{1}HNMR$ 7.26-8.45 (m,9H,Ar-H), 8.81 (s,1H,NH), 8.36 (s,1H,CH=N). (IIId) $^{1}HNMR$ 5.87(s,1H,OH), 9.02 (s,1H,NH), 7.39-8.51 (m,4H,Ar-H ,benzothiazole), 6.83- 8.05(m,5H,Ar-H ,benzyliden). (IIIe) $^{1}HNMR$ 9.55 (s,1H,NH), 8.48 (s,1H,N=CH), 7.13-7.96 (m,8H,Ar-H). (IVe) $^{13}CNMR$, 183.47 (1C,S-C=N), 129.31, 131.29,(2C, 1,4-disub.benzene), 14 signal 112.25-146.72 (C of Ar-C), 160.38(1C,imine), (Ve)7.41 (s,1H,NH) , 7.69-8.93 (m,12H,ArC-H). (VIb) 7.67 (s,1H,NH) , 7.82-8.95 (m,12H,ArC-H), (VIb) $^{13}CNMR$ 115.25,117.89(1C,=CH benzothiazole),120.87, 122.17,122.32,124.04(2C,=CH benzene 1,4-disubstitution), 8 signals at 126.52-167.82(1C,C-Cl, =C-S, N=C-N, C-Cl, N-C=Ar, =C-N, N=C-N , N=C(S)-N) [(24)].

Pharmacology:

All the newly synthesized substituted formazan derivatives (IV,V,VI) were tested for their antibacterial activity in vitro against *Staph. aureus, E. coli, Sal. typhi, Ps. aeruginosa* . The results are summarized in Table-5 with ampicillin and erythromycin as standard for comparison. Among them, the formazans highest exhibitionand selectivity with E.coli germ , the others germ was showed moderate inhibitory activity, see Table-5.

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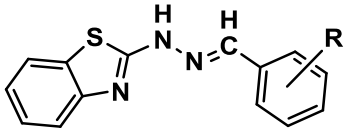
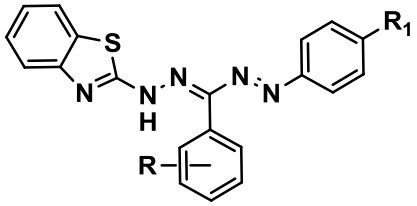
Table-1: Physical data of aryl substituted (benzothiazol-2-yl) hydrazone (IIIa-i)



Comp . No.	R	Colour	Molecular formula	R_f	M.P	Yield %		Rec.Solv.
						Solvent	µW	
IIIa	H	Pale Yellow	$C_{14}H_{11}N_3S$	72	226 -228	84	88	Ethanol
IIIb	2-OH	Pale Yellow	$C_{14}H_{11}N_3OS$	65	188 – 190	82	91	Methanol
IIIc	3-OH	Yellowish white	$C_{14}H_{11}N_3OS$	86	244 - 246	85	93	Ethanol
IIId	4-OH	Dark Yellow	$C_{14}H_{11}N_3OS$	80	248 – 250	80	92	Ethanol
IIIe	2-Br	Pale Yellow	$C_{14}H_{10}BrN_3S$	77	244 – 246	92	94	Methanol
IIIf	4-Cl	Pale Yellow	$C_{14}H_{10}ClN_3S$	76	263 – 265	75	87	Ethanol
IIIg	3-OCH3	Dark Yellow	$C_{15}H_{13}N_3OS$	85	233 – 235	78	83	Ethanol
IIIh	4-$N(CH_3)_2$	Yellow	$C_{16}H_{16}N_4S$	75	230 - 232	90	92	Ethanol
IIIi	3-NO2	Dark Yellow	$C_{14}H_{10}N_4O_2S$	90	256 – 258	81	92	Ethanol

Table-2:Physical data of 1-(benzothiazol-2-yl)-5-(4-chlorophenyl)-3-arylformazan

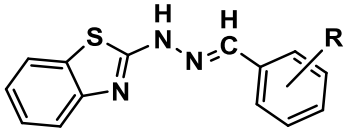


Comp . No.	R	R_1	Colour	Molecular Formula	R_f	M.P	Yield %		Fixed Colours (in paint)			Rec.Solv.
							Solvent	µW	C	Wl	Wwd	
IVa	3-OCH_3	-Br	Brown	$C_{21}H_{16}BrN_5OS$	72	110-112	75	87	Dark Brown	Brown	Pale Brown	Ethanol
IVb	3-NO_2	=	Red-Brown	$C_{20}H_{13}BrN_6O_2S$	65	Gum	82	90	Brown	Red-Brown	Red	Methanol
IVc	4-$N(CH_3)_2$	=	Brown	$C_{22}H_{19}BrN_6S$	86	Gum	84	92	Brown	Brown	Brown	Ethanol
IVd	-H	=	Brown	$C_{20}H_{14}BrN_5S$	75	110-112	85	89	Brown	Brown	Brown	Ethanol
IVe	2-OH	=	Brown	$C_{20}H_{14}BrN_5OS$	90	Gum	90	91	Brown	Brown	Brown	Methanol
Va	3-OH	-NO_2	Light Brown	$C_{20}H_{14}N_6O_3S$	76	Gum	82	92	Brown	Light Brown	Pale Brown	Ethanol
Vb	4-$N(CH_3)_2$	=	Brown	$C_{22}H_{19}N_7O_2S$	73	Gum	78	92	Brown	Brown	Brown	Ethanol
Vc	4-Cl	=	Brown	$C_{20}H_{13}ClN_6O_2S$	78	Gum	80	92	Brown	Brown	Brown	Ethanol
Vd	4-OH	=	Brown	$C_{20}H_{14}N_6O_3S$	90	Gum	81	94	Brown	Brown	Brown	Ethanol
Ve	2-Br	=	Brown	$C_{20}H_{13}BrN_6O_2S$	84	Gum	80	91	Brown	Pale	Pale	Methanol

										Brown	Brown	
VIa	3-NO_2	-Cl	Dark Brown	$C_{20}H_{13}ClN_6O_2S$	81	Gum	79	88	Brown	Brown	Brown	Ethanol
VIb	4-Cl	=	Brown	$C_{20}H_{13}C_{l2}N_5S$	88	228-230	79	90	Brown	Brown	Pale Brown	Ethanol
VIc	-H	=	Brown	$C_{20}H_{14}ClN_5S$	76	Gum	85	92	Brown	Pale Brown	Pale Brown	Methanol
VId	2-OH	=	Brown	$C_{20}H_{14}ClN_5OS$	79	102-104	84	95	Brown	Brown	Brown	Ethanol
VIe	3-OH	=	Brown	$C_{20}H_{14}ClN_5OS$	86	102-106	82	90	Red-Brown	Red-Brown	Pale Red	Ethanol

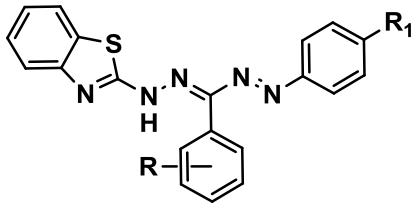
C: cotton , Wl: wool , Wwd: white wood powder

Table-3: Spectral data for the synthesized compounds(IIIa-i)



Comp. No.	R	UV,λmax (nm), EtOH π→π* n→π*	IR , (KBr), cm^{-1}								
			Fixed bands in structure							Changed bands in structure	
			ν N – H	ν(=CH)Ar	νC – H Aliph	ν C=N.	νC=CAr	ν C – N	ν N- N		
IIIa	H	240 294	3413	3076	2960,2885	1627	1575,1440	1367	1022	-----	-----
IIIb	2-OH	213 308	3230	3100	2975,2880	1614	1512, 1442	1367	1024	ν (O – H),3448	ν (C–OH),1164
IIIc	3-OH	223 357	3224	3082	2958,2879	1623	1575, 1492	1365	977	ν(OH),3407	ν (C–OH),1157
IIId	4-OH	228 304	3261	3099	2975,2925	1608	1510, 1446	1365	1008	ν(OH),3409	ν (C–OH),1164
IIIe	2-Br	302 348	3411	3060	2945,2852	1616	1573, 1473	1361	1020	ν (C – Br),617	-----
IIIf	4-Cl	241 289	3191	3074	2962,2887	1625	1575, 1444	1313	1010	ν (C – Cl), 1091	-----
IIIg	3-OCH_3	279 321	3190	3076	2881,2802	1610	1521, 1440	1353	941	ν (C–O–C),1180	-----
IIIh	4-$N(CH_3)_2$	225 348	3188	3074	2960,2883	1608	1521, 1440	1355	943	-----	-----
IIIi	3-NO_2	281 302	3191	3074	2914,2875	1622	1529, 1444	1309	999	ν(NO_2)1575	-----

Table-4: Spectral data for the synthesized compounds(IVa-e,Va-e,VIa-e)



Comp. No.	R	R1	UV,λmax (nm), EtOH π→π* n→π*	IR , (KBr), cm^{-1}							
				Fixed bands in structure						Changed bands in structure	
				ν N – H ν(=CH)Ar	νC – H Aliph	ν C=N.	νC=CAr	ν C – N	ν N =		
IVa	3-OCH_3	-Br	230 282	3188 3074	2958,2885	1608	1573	1267	1521	ν (C–O–C),1180	-----
IVb	3-NO_2	=	232 327	3205 3049	2955,2808	1631	1602	1244	1525	ν(NO2),1461,1348	ν (C – Br),613
IVc	4-$N(CH_3)_2$	=	222 343	3205 3051	2945,2884	1633	1601	1243	1524	-----	ν (C – Br),617
IVd	-H	=	231 323	3448 3053	2956,2852	1622	1542	1270	1525	ν (C – Br),613	-----
IVe	2-OH	=	278 337	3429 3053	2997,2837	1606	1587	1247	1527	ν(OH),3429	ν (C – Br),615
Va	3-OH	-NO_2	237 326	3205 3051	2939,2845	1631	1600	1242	1527	ν(OH),3406	ν(NO_2),1479,1340
Vb	4-$N(CH_3)_2$	=	285 351	3252 3062	2976,2857	1628	1607	1243	1524	ν(NO_2),1523,1354	-----
Vc	4-Cl	=	241 377	3124 3051	2999,2854	1631	1602	1244	1527	ν(NO_2),1481,1348	ν (C – Cl),1054
Vd	4-OH	=	283 372	3205 3051	2939,2858	1631	1604	1244	1527	ν(OH),3446	ν(NO_2),1479,1342
Ve	2-Br	=	270 362	3410 3048	2986,2871	1621	1607	1243	1526	ν (C – Br),617	-----
VIa	3-NO_2	-Cl	273 357	3278 3040	2953,2859	1635	1603	1241	1527	ν(NO_2),1531,1347	ν (C – Cl),1080
VIb	4-Cl	=	219 343	3206 3053	2969,2848	1625	1604	1256	1528	ν (C – Cl),1090	-----
VIc	-H	=	213 309	3290 3062	2971,2859	1632	1600	1247	1524	ν (C – Cl),1078	-----
VId	2-OH	=	245 368	3403 3080	2982,2844	1628	1602	1251	1524	ν(OH),3435	ν (C – Cl),1091
VIe	3-OH	=	212 353	3212 3059	2973,2851	1630	1601	1244	1527	ν(OH),3445	ν (C – Cl),1086

Table-5: Antimicrobial activity for some prepared compounds .

Comp. No.	*Staph. aureus*	*E. coli*	*Sal. typhi*	*Ps. aeruginosa*
IVa	±	+	-	-
IVb	±	+	±	-
IVc	±	±	+	+
IVd	-	++	±	-
IVe	±	±	+	-
Va	-	-	-	±
Vb	±	±	+	-
Vc	±	+	±	±
Vd	-	±	±	-

Ve	±	+	±	-
VIa	±	+	±	±
VIb	±	+	±	±
VIc	+	++	±	±
VId	-	±	±	-
VIe	±	+	±	-
Blank disk	-	-	-	-
Ampicillin	±	+	-	-
Erythromycin	±	±	±	-

Key the symbols: (-) = no inhibition, (±)= 6-9mm, (+)= 10-14mm, (++) = 15-22mm.

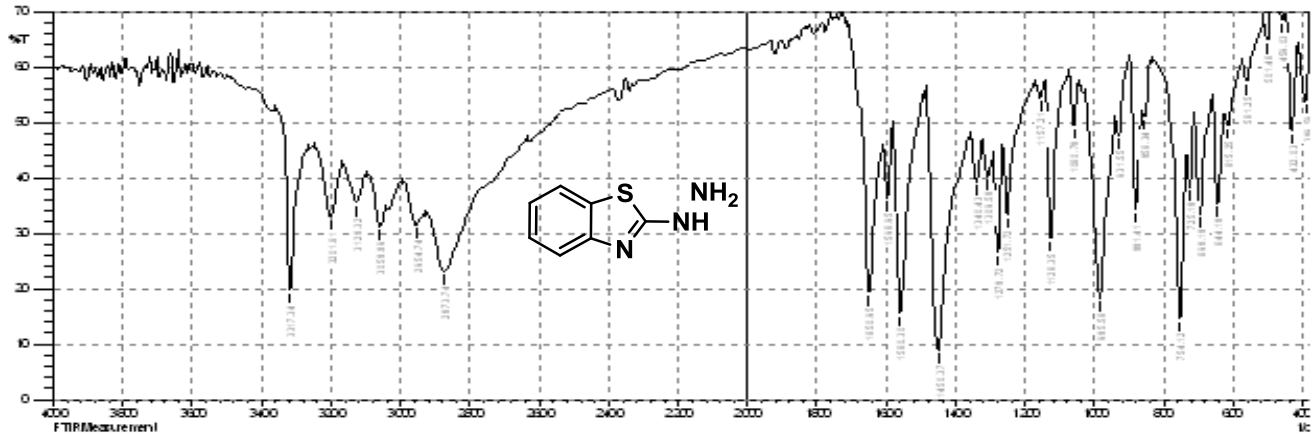


Fig-1: FT-IR spectrum of the compound (II)

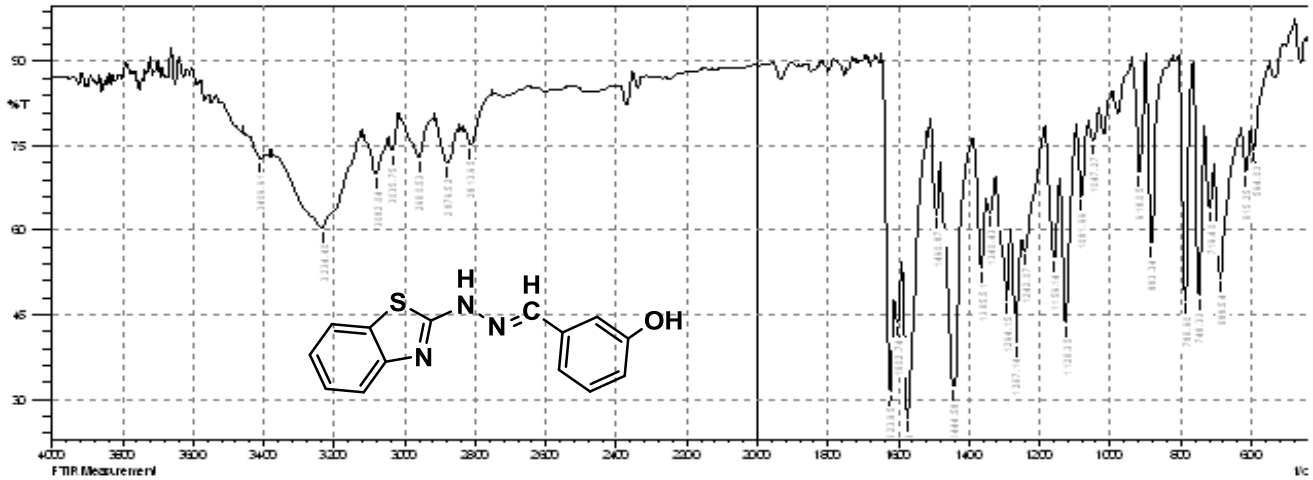


Fig-2: FT-IR spectrum of the compound (IIIc)

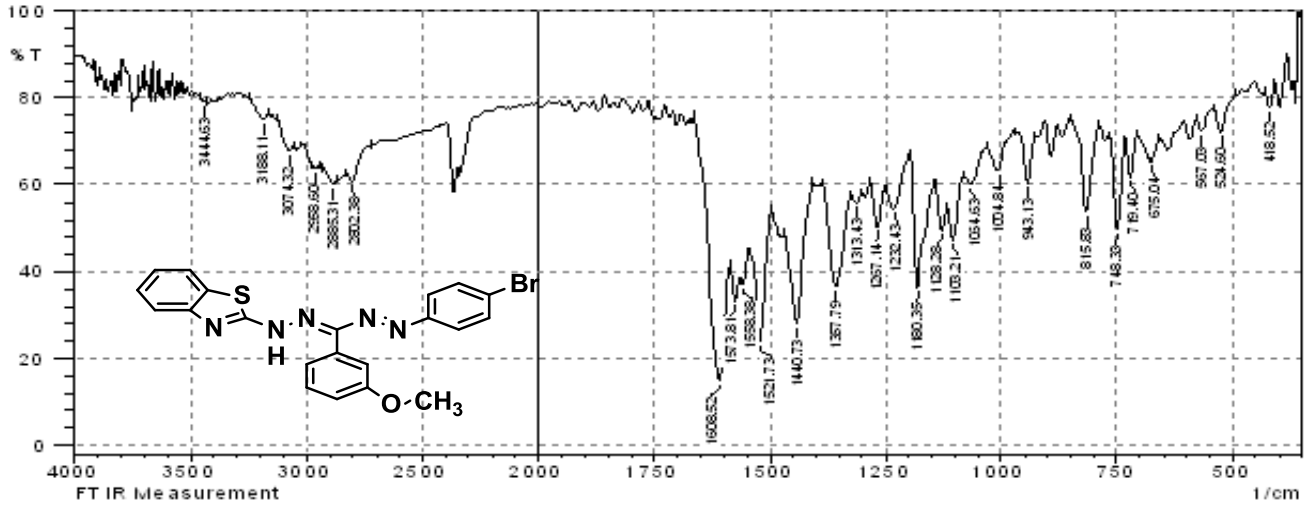


Fig-3: FT-IR spectrum of the compound (IVa)

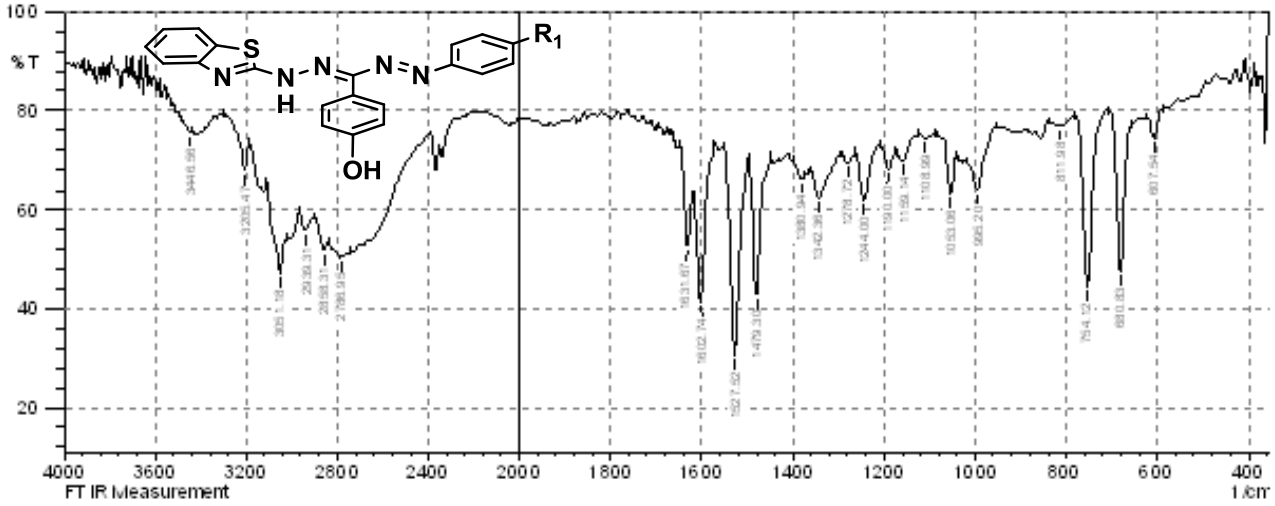


Fig-4: FT-IR spectrum of the compound (Vd)

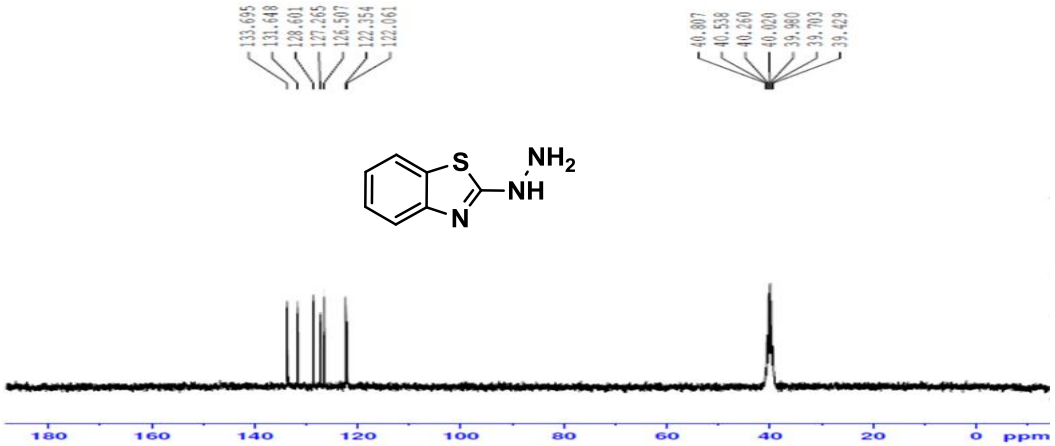


Fig-5: ^{13}CNMR spectrum of the compound (II)

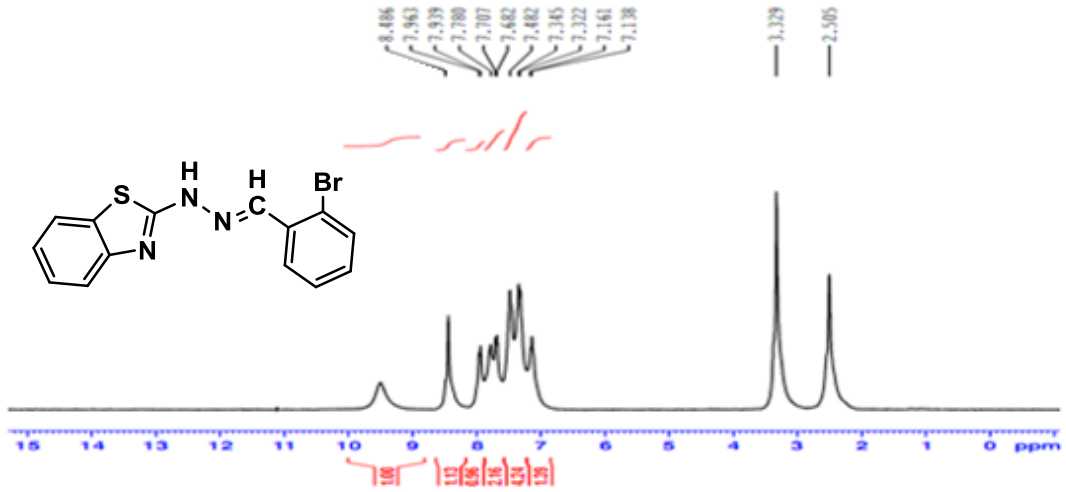


Fig-6: ^{1}HNMR spectrum of the compound (IIIe)

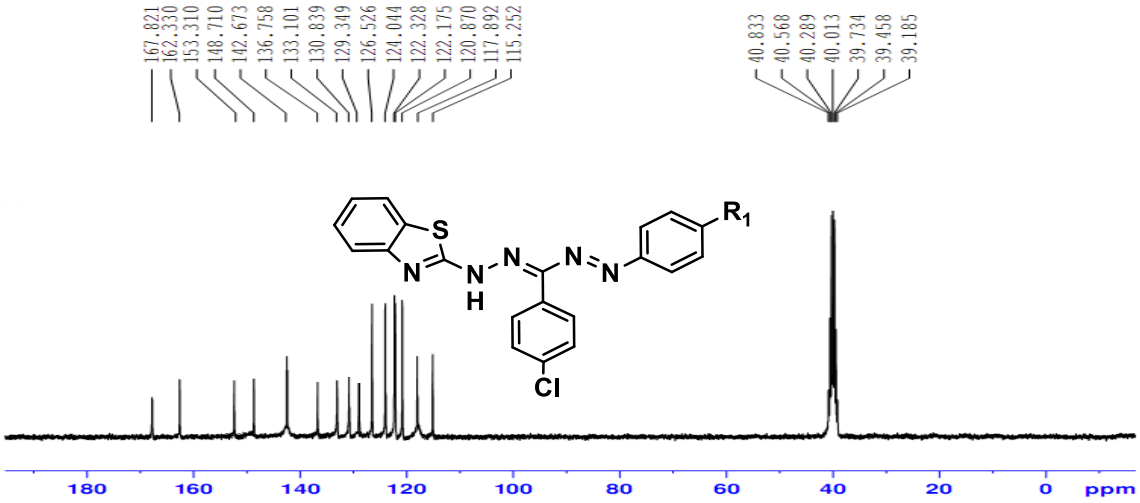


Fig-7: ^{13}CNMR spectrum of the compound (VIb)