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SYNTHESIS, IDENTIFICATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF SOME NEW N-SUBSTITUTED-2-(METHYLTHIO) BENZIMIDAZOLE CONTAINING HETEROCYCLIC RING

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ABSTRACT
New derivatives of 1,2,3-triazole and pyrazole were obtained from the work that was conducted in this research. Firstly; reaction of 2-mercaptobenzimidazole (2-MBI) in a basic condition with methyl iodide gave 2-(methylthio)-1H-benzo[d]imidazole [I] then compound [I] was reacted with sodium hydride in DMF at (0 °C) to bring forth the salt of compound [1]; subsequently, the produced salt was reacted with chloroacetylchloride to produce 2-chloro-1-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)ethanone as starting compound [II] . Thereafter, reaction of compound [II] was carried out through two pathways: first pathway, involved a reaction with sodium azide to give 2-azido-1-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)ethanone compound [III] which was entered in 1,3-dipolar cycloaddition with α,β- unsaturated carbonyl compounds [a-e] to give 1,2,3-triazole derivatives [IV_{a-e}]. While the second pathway was achieved by reaction with *p*-phenylenediaimine to give compound [V] then diazoniation to produce diazonium salt which was, in turn, coupled with acetylacetone to give compound [VI]. Subsequently, the product of the previous reaction was reacted with hydrazine derivatives [a-e] to give pyrazole derivatives [VII_{a-e}]. The new prepared compounds were identified by [FTIR, ^{1}H-NMR and ^{13}C-NMR] and their physical properties were measured. Furthermore, we have evaluated the effect of some prepared compounds on some bacterial and fungal strains.

KEYWORDS: 2-MBI, 2-(methylthio) benzimidazole, 1,2,3-triazole, pyrazole, anti-microbial.

INTRODUCTION

Heterocyclic compounds constitute a key-component in numerous natural products, to name a few; vitamins, hormones, alkaloids, a wide range of antibiotics, pharmaceutical products, herbicides, anti-aging medicines and plenty other industrial products of high importance (different types of dyes, corrosion inhibitors, stabilizing agents, sensitizers, etc) [(1)]. In fact, more than half of all known organic compounds are heterocycles [(2)]. So far, 2-Mercaptobenzimidazoles are the most frequently encountered heterocyclic in medicinal and industrial chemistry [(3)] with a wide variety of essential biological activities such as anti-histamine, anti-microbial, neutropic and analgesic activities [(4)]. 2-Mercaptobenzimidazole compounds contain both -NH and -SH groups and alkylation was noticed to be more inclined to occur on sulphur rather than nitrogen due to higher neucleophiliticy of sulphur in comparison with nitrogen [(5)]. Moreover; alkylation of 2- Mercapto benzimidazole under basic conditions using different alkyl halides and aryl halides give the thio ether derivatives [(6)]. On other hand, Triazoles are five-membered heterocyclic compounds which contain three nitrogen atoms [(7)]. Different synthetic methodologies have been utilized recently to prepare Triazole ring system 1,3-Dipolar cycloaddition reactions of azides with (alkyne [(8)], α, β-unsaturated ketones with azides [(9)] and from arylglyoxaldoxime) [(10)]. Many 1, 2, 3-triazoles are found to be potent anti-microbial [(11)], anti-inflammatory [(12)], anti-malarial [(13)], anti-viral agents [(14)] and a unit of high significance with anticancer profile in many of the human cell lines [(15)]. Pyrazoles are heterocyclic organic compounds characterized by a five-membered ring of three carbon atoms and two adjacent nitrogen centers [(16)]. Generally speaking, pyrazoles are synthesized by reaction of 1,3-diketones with hydrazine, 1,3-dipolar cycloaddition of diazo compounds with alkynes and reaction of α,β-unsaturated aldehydes and ketones with hydrazine[(17)]. Interestingly enough; a large number of pyrazoles have been produced and some of them gained applications on the clinical level as well. These compounds have diverse biological activities such as; anti-microbial [(18)], anti-cancer [(19)], anti-inflammatory [(20)], anti-microbial and anti-oxidant [(21)]. In this research we aimed to synthesize new 2-chloro-1-(2-(methylthio)-1H-benzo[d]imidazol-1-yl)ethanone derivatives including 1,2,3-triazole and pyrazole moieties and evaluation of its' antimicrobial activities.

MATERIALS & METHODS

All chemicals used were supplied by: Merck, BDH, Fluka and sigma Aldrich chemicals companies. The melting point was recorded using Gallenkamp, electro-thermal melting point apparatus. Infrared spectra were recorded using (FTIR) 8400s Fourier transitions infrared spectrometer shimadzu, Japan, (KBr) disc in (4000-600) cm^{-1} spectral range, in the Department of Chemistry, College of Science, University of Baghdad and Research Laboratory, College of Pharmacy, University of Al-Mustansiriyah. ^{1}H-NMR & ^{13}CNMR spectra were

recorded on near magnetic resonance Bruker, Ultra-shield (400) MHz, in the University of Ain-Shams, College of Pharmacy, Egypt. Also; DMSO-d*6* was used as a solvent and Central Laboratory Isfahan University, Iran. $CDCl_3$ was used as solvent.

Synthesis of 2-(methylthio)-1H-benzo[d]imidazole [I]

This compound was prepared according to the literature's procedure. [6]

Synthesis of 2-chloro-1-(2-(methylthio)-1H-benzo-[d] imidazol-1-yl)ethanone [II] [22]

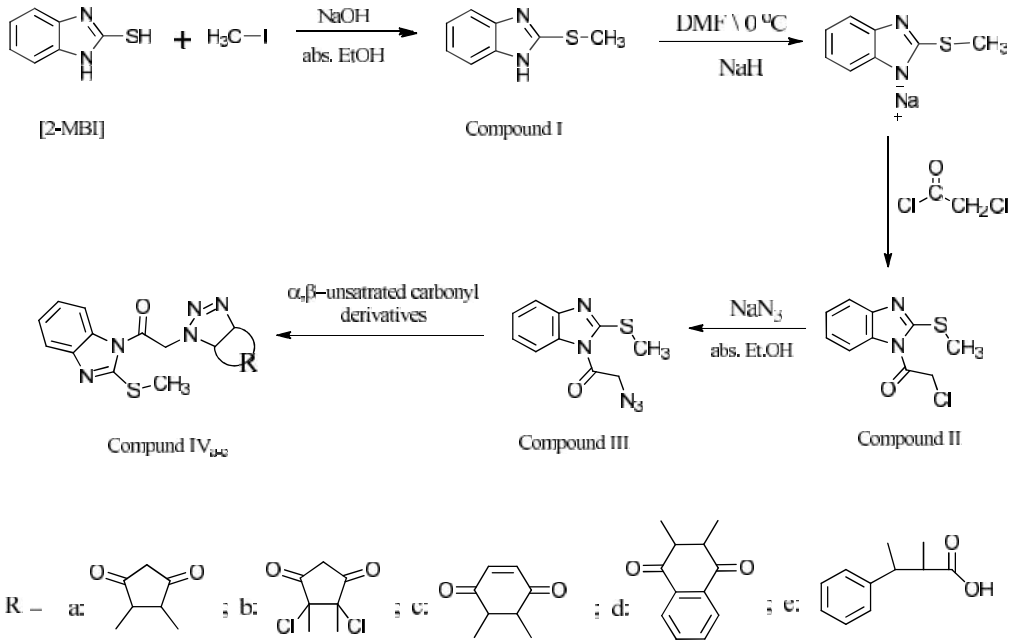
Solution of compound [I] (1gm, 0.006 mole) in anhydrous dimethyl formamide (DMF) (7ml) was cooled to 0°C, and Sodium hydride (0.14gm, 0.006 mole) in small a portions was added. The solution was stirred for (30 minutes) then Chloroacetyl chloride (0.5ml, 0.006mole) was added drop wise. The mixture was stirred for 10 minutes at 0°C, and then stirred at room temperature for (4 hours). The solvent was evaporated then poured into ice water and filtered. The precipitate was recrystallized with ethanol to give a brown powder.

Synthesis of 2-azido-1-(2-(methylthio)-1H-benzo[d] imidazol-1-yl)ethanone [III] [23]

Consequently, Sodium azide (0.52gm, 0.008 mole) was added to the solution of compound [II] (1gm, 0.004 mole) in absolute ethanol (10ml). The mixture was refluxed for (6 hours) with stirring. The solvent was evaporated then light brown precipitate was washed with cold water, filtrated and recrystallized with ethanol.

General Procedure for Synthesis of 2-(methylthio)-1H-benzo[d]imidazole triazole moiety derivatives [IVa-e]: [23]

Afterwards, α, β-unsaturated carbonyl compounds (0.001 mole) was added to the solution of compound [III] (0.25gm, 0.001 mole) in absolute ethanol (10ml). The mixture was refluxed for (24 hours). The solvent was evaporated and the residue was washed with diethyl ether. As shown in Scheme 1.



SCHEME 1: Shows the synthesis of the target compounds (IV_{a-e})

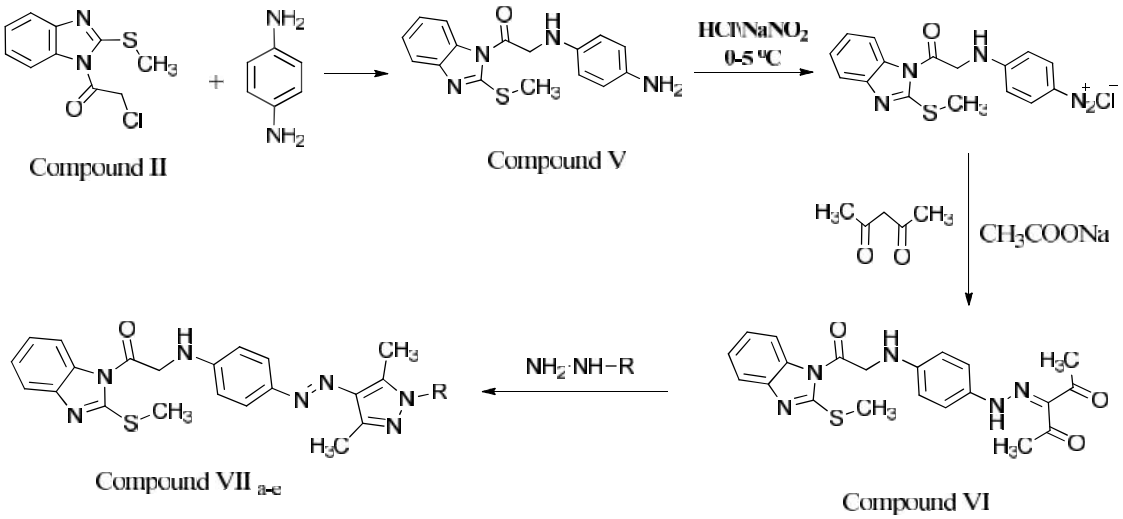
Synthesis of 2-((4-aminophenyl)amino)-1-(2-(methyl thio)-1H-benzo[d]imidazol-1-yl)ethanone [V] [24]

Compound [II] (0.72gm, 0.003 mole) was dissolved in absolute ethanol (10ml) then anhydrous potassium carbonate (0.41gm, 0.003 mole) was added. The mixture was stirred then *p*-phenylenediamine (0.32gm, 0.003 mole) solution in absolute ethanol (10ml) was added drop-wise. The mixture was refluxed and stirred for (8 hours). The solvent was evaporated and the product was washed with cold water, filtrated and recrystallized with (ethanol-water).

Synthesis of 3-(2-(4-((2-(2-(methylthio)-1H-benzo[d] imidazol-1-yl)-2-oxoethyl) amino) phenyl) hydrazono) pentane-2,4-dione [VI] [25]

Compound [V] (0.31gm, 0.001 mole) was dissolved in concentrated HCl (2ml) and cooled in an ice bath at 0-5 °C, then drop-by-drop solution of Sodium nitrite (0.07gm, 0.001 mole) in (5ml) water was added to it, stirred for (30minutes) and the temperature was maintained at 0-5 °C. The mixture was added drop-by-drop to Acetyl acetone (0.1gm, 0.001 mole), Sodium acetate (0.16gm, 0.002 mole) in absolute ethanol (5ml) solution. The mixture was stirred for (30 minutes). Then after the solvent was left to evaporate, the product was recrystallized with ethanol.

General Procedure for Synthesis of 2-(methylthio)-1H-benzo[d]imidazole pyrazole moiety derivatives (VIIa-e): [26] Hydrazine derivatives compounds (0.0006mol) were added to compound [VI] (0.25gm, 0.0006mol) in absolute ethanol (10ml) solution. The mixture was stirred and refluxed for (10-12 hours), the solvent was evaporated and the product was washed with water then diethyl ether. As shown in Scheme 2.



SCHEME 2. Shows the synthesis of the target compounds (VII $_{a-e}$)

Anti-microbial activity test: [27,28]

Some of the prepared compounds were tested clinically against two strains of bacteria (+ve) bacterial isolates as: *Streptococcus faecalis* from the (ear) and *Staphylococcus aureus* from the (wound) and two strains from clinical gram (–ve) bacterial isolates as: *Escherichia coli* from (urine) and *Klebsiella pneumonia* from the (ear) in addition to *Candida albicans* from (vagina) as fungus isolate. The anti-microbial activities of each derivative compounds were studied by using agar well diffusion method, which used Mueller Hinton Agar (MHA) and Blood Agar Base (BAB). DMSO used as the negative controller and Ciprofloxacin was used as a standard drug for antimicrobial. All tests were performed in college of Pharmacy / university of Al-mustansiriyah. The results are displayed in Table 1.

TABLE 1: antimicrobial activity of synthesized compounds

Sample code and standard	Concentration (µg/ml)	Zone of inhibition (mm)				
		Gram positive		Gram negative		Fungal
		Strepto.	Staphylo.	*E. coli*	Klebsiella	Candida al.
IV_a	800	-	12	2	-	2
	400	-	10	18*	-	4
IV_b	800	-	14	18*	-	6
	400	-	14	12*	-	4
IV_c	800	-	10	8	-	4
	400	-	10	4	-	2
IV_d	800	-	12	4	-	4
	400	-	12	4	-	2
IV_e	800	6	12	4	-	4
	400	4	12	4	-	6
VII_a	800	4	20*	6	-	8
	400	6	12	4	-	6
VII_b	800	4	12	6	-	4
	400	4	14	4	-	2
VII_c	800	4	12	4	-	-
	400	4	14	4	-	4
VII_d	800	2	12	12*	-	18*
	400	4	14	6	-	18*
VII_e	800	4	12	6	-	6
	400	4	10	4	-	6
Ciprofloxacin	10	40	16	-	30	12
DMSO	-	-	-	-	-	-

Zone of inhibition: (-) no inhibition; (3-6) weak; (7-10) moderate; (11-15) strong

RESULTS & DISCUSSION

Synthesis of the target compounds (IV_{a-e} & VII_{a-e}) were detected by spectral (FTIR, ^{1}HNMR & ^{13}C-NMR), sharped band in compound (II) in 1718 cm^{-1} that belongs to amide group because we have α-chloro to amide group that increased the frequency [29]. While, in (^{1}HNMR) the chemical shift disappeared at 12.5 ppm that belongs to (NH) and in (^{13}C-NMR) the signal appeared at 173 ppm for (C=O) amide as show in tables (2-3).

On other hand, the absorped band in 1437-1446 cm^{-1} in compounds (IV_{a-e}) belongs to N=N group of triazole and disappeared at 2129 cm^{-1} for azide group in compound [III]. In pyrazole compounds (VII_{a-e}) pathway synthesis, we have absorption band in compound (V) at 3369 (asym.) and (3323 sym.) for (NH_2) while in (^{1}HNMR) we have signal at 6.5 ppm for (NH_2) and 7.43 ppm for (NH) as show in tables (4-5). In compound (VI) was disappeared (NH_2) band in (FTIR) and signal in (^{1}H-NMR). The presence band in compound (VIIa-e) at 1435-1460 cm^{-1} which indicate the formation N=N and disappeared 1730 cm^{-1} that belonged to C=O of diketone in compound [VI] as show in tables (6-7). Table 1 shows the inhibition zones in (mm) of the mentioned derivatives. In comparison with standard drug (Ciprofloxacin), the antimicrobial activity showed that compounds (IVa-e & VIIa-e) were specific for *Escherichia coli* bacteria while showed no activity for *Klebsiella pneumonia* while in *Streptococcus faecalis* bacterial showed weak activity. Some of the prepared compounds showed high activity for *Staphylococcus aureus* bacteria and *Candida albicans* fungi.

TABLE 2: physical properties and FTIR spectral data cm^{-1} of compounds [2-MBI, I, II]

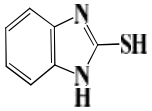
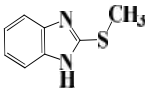
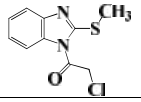
Comp. NO.	Structure	Melting Point (C°)	Yield %	FTIR spectral data cm^{-1}					
				ν(N-H)	ν(C-H) aroma.	ν(C-H) alipha.	ν(C=C) aroma.	ν(C=O) amide	Others
2-MBI		300-304	-	3155	3050	-	1465	-	ν(C=N Imidazole) 1622 ν(SH) 2764
I		199-200	77	3220	3053	2960 2872	1465 1500	-	ν(C=N Imidazole) 1620 ν(C-S-C) 665
II		122-124	85	-	3061	2955 2924 2854	1570 1502	1718	ν(C=N Imidazole) 1616 ν(C-Cl) 794

TABLE 3: ^{1}HNMR and ^{13}CNMR spectral data (δ ppm) of compounds [I,II]

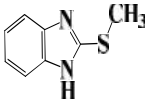
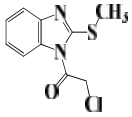
Com. No.	Structure	^{1}HNMR spectral data (δ ppm)	^{13}CNMR spectral data (δ ppm)
I		2.70 (s,3H,-CH_3); 7.09-7.43 (m,4H,Ar-H); 12.68 (s,H,-NH)	-
II		2.90 (s,3H,S-CH_3); 4.74 (s,2H,-CH_2-Cl); 7.41-7.73 (m,4H, Ar-H)	40.81 (S-CH_3); 70.79 (CH_2); 113-139 (Ar); 156.33 (N=C-N); 173.60 (O=C-N)

TABLE 4: physical properties and FTIR spectral data cm^{-1} of compounds [III,IVa-e]

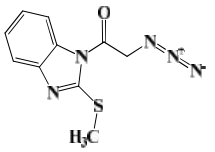
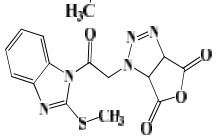
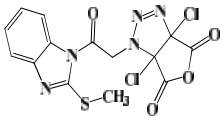
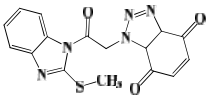
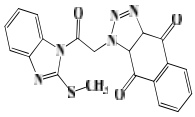
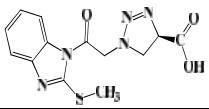
Com. NO.	Structure	Melting Point (C°)	Yield %	FTIR spectral data cm^{-1} ν(C-H) aroma.	ν(C-H) alipha.	ν(C=N)	ν(C=C)	ν(C=O)	Others
III		182-184	80	3053	2954 2924 2808	1616	1589-1498	1710	**ν(N_3)** 2129 1437
IV_a		98-100	70	3053	2990 2958 2908	1625	1550-1450	1856 1781 **(anhyd.)** 1710 **(amide)**	**ν(N=N)** 1446 **ν(C-O-C)** 1279
IV_b		90-92	65	3063	2960 2918 2841	1620	1593 1525	1800 1726 **(anhyd.)** 1680 **(amide)**	**ν(C-Cl)** 1018 **ν (N=N)** 1446 **ν(C-O-C)** 1259
IV_c		118-120	89	3055	2956 2924 2854	1612	1591-1508	1731 **(ketone)** 1700 **(amide)**	**ν(N=N)** 1437
IV_d		114-116	95	3057	2962 2926 2862	Overlap with amide	1589-1496	1731 **(ketone)** 1684 **(amide)**	**ν(N=N)** 1438
IV_e		122 (d.)	90	3053	2956 2924 2854	1633	1550-1498	1720 **(acid)** 1687 **(amide)**	**ν(N=N)** 1440 **ν(OH acid)** 3300-2700

TABLE 5: ^{1}HNMR and ^{13}CNMR spectral data (δ ppm) of compounds [IV_b,IV_d]

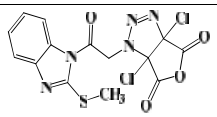
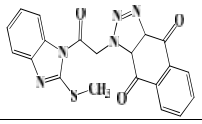
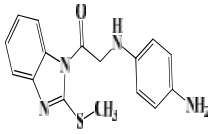
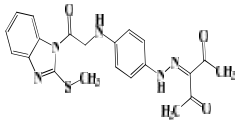
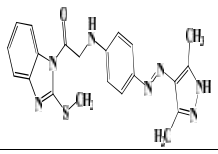
Com. NO.	Structure	^{1}HNMR spectral data (δ ppm)	^{13}CNMR spectral data (δ ppm)
IV_b		2.70 (s,3H,-S-CH_3); 3.39 (s,2H,-CH_2-N); 7.08-7.49 (m,4H,Ar-H)	40.39 (CH_3); 78.27 (CH_2); 103.11 (C-Cl); 115.58-139.39 (Ar); 150.53 (N=C-N); 176.48 (O=C-N); 189.76 (O=C-O)
IV_d		2.70 (s,3H,-S-CH_3); 3.39 (s,2H,-CH_2-N); 5.77 (s,H,CH-quinone ring); 7.11-7.98 (m,8H, Ar-H)	40.39 (CH_3); 78.34 (CH_2); 85.56 (CH-N); 121.26 (Ar); 151.56 (N=C-N); 175.18 (O=C-N); 194.66 (C=O diketone)

TABLE 6: physical properties and FTIR spectral data cm^{-1} of compounds [V-VII_{a-e}]

Com. NO.	Structure	Melting Point (C°)	Yield %	FTIR spectral data cm^{-1} ν(NH)	ν(C-H) aroma.	ν(C-H) alipha.	ν(C=N)	ν(C=O)	Others
V		102-104	68	3215	3059	2926 2816	1618	1700	**ν(p-NH_2)** 831 **ν(NH_2)** 3369 (asy.) 3323 (sym.) **ν(C=Caro.)** 1600-1552
VI		78-80	93	3311	3058	2958 2924 2858	1624	1730 **(ketone)** 1700 **(amide)**	**ν(C=C aro.)** 1589-1512
VII_a		81-83	80	3325	3049	2960 2949 2864	1624	1700	**ν(N=N)** 1435

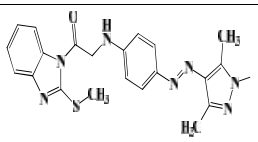
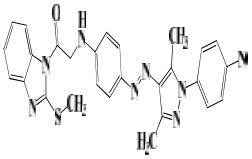
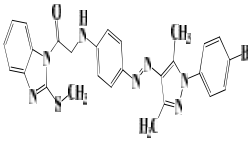
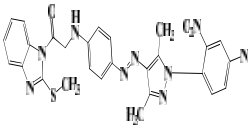
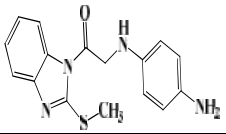
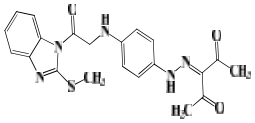
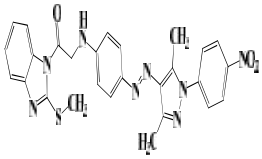
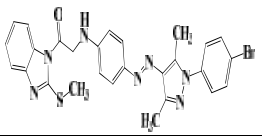
VII_b		70-72	69	3211	3039	2955 2856	1656	1705	**ν(N=N)** 1435
VII_c		110 (d.)	75	3193	3099	2923 2854	1623	1701	**ν(NO_2)** 1517 (asy.) 1344 (sym.) **ν (N=N)** 1458
VII_d		160 (d.)	85	3342	3060	2962 2925 2864	1655	1696	**ν(C-Br)** 594 **ν(N=N)** 1438 **ν(p-Br)** 648
VII_e		148 (d.)	86	3325	3097	2928 2852	1641- 1616	1700	**ν(NO_2)** 1514 (asy.) 1330 (sym.) **ν(N=N)** 1460

TABLE 7: ^{1}HNMR and ^{13}CNMR spectral data (δ ppm) of compounds [V,VI,VII_c, VII_d]

Com. NO.	Structure	^{1}HNMR spectral data (δ ppm)	^{13}CNMR spectral data (δ ppm)
V		2.51 (s,3H,-CH_3); 3.35 (d,2H,-CH_2); 6.50 (s,2H,-NH_2); 7.09-7.11 (m,4H,Ar-H); 7.43 (t,H,-NH)	40.61 (CH_3); 65 (CH_2); 121.71 (Ar); 151.65 (N=C-N); 174.3 (O=C-N)
VI		2.63 (s,3H,S-$\underline{CH_3}$); 2.76 (s,6H, $\underline{CH_3}$-C=O); 3.05 (d,2H,-CH_2); 5.95 (s,H,-$\underline{NH}$-N=C); 7.39-7.76 (m,8H, Ar-H); 7.83 (t,H,-$\underline{NH}$-CH_2)	-
VII_c		2.01 (s,6H,-C=C-$\underline{CH_3}$); 2.98 (s,-S-CH_3), 4.27 (t,2H,-$\underline{CH_2}$-NH); 7.44-7.82 (m,12H,Ar-H); 8.40 (t,H,-CH_2-$\underline{NH}$)	-
VII_d		2.11 (s,6H,-C=C-$\underline{CH_3}$); 2.83 (s,3H,-S-CH_3); 3.88 (t,2H,-$\underline{CH_2}$-NH); 7.33-7.53 (m,12H,Ar-H); 8.30 (t,H,-CH_2-$\underline{NH}$)	-

CONCLUSION

This work demonstrates the reactions involved in the synthesis of new heterocyclic compounds and hence, the detection of their antimicrobial activity. The antimicrobial activity of these compounds was evaluated against Gram-positive, Gram-negative bacteria and fungi. The target compounds (IVa-e & VIIa-e) showed more significant antimicrobial activity than a standard well-known drug. On the other hand, most of the compounds showed a moderately significant antimicrobial activity.

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