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SYNTHESIS AND ANTI-TUBERCULAR ACTIVITY OF SOME 1,2,4-TRIAZOLE DERIVATIVES

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Abstract:

The synthesis of a number of bis-triazole derivatives starting from 2,2`-bis-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)diphenyl amine (**I**) was achieved . Reactions involving its two functional groups for structural diversity was performed. Thus reacting **I** in DMSO, in CS_2/KOH, with acetic anhydride, chloroacetyl chloride, ethyl chloroacetate, epichlorohydrin, 2-chloro-5-nitropyridine, certain-2-chloro aromatic acids (alone or in presence of $POCl_3$), various α,β-unsaturated ketones and different aldehydes afforded **II-XII** respectively. Compounds $\mathbf{XII}_{(a-h)}$ were further cyclised in nitrobenzene to yield substituted bis-triazolothiadiazoles ($\mathbf{XIII}_{a-h}$).

Compounds **I-III, V, VIII, XI, XII, XIII** were tested for their anti-tubercular activity. Compounds **XIc** and **XIIIb** were found to be active.

INTRODUCTION

Several bis-compounds having a diverse range of structures and pharmacological activities were synthesized and reported[1-18] to have better pharmacodynamic or pharmacokinetic properties, compared to the monomers. Ethambutol is a symmetrical diamine (bis-compound), which is used routinely in the clinical treatment of TB. Also, a second generation of ethambutol analogues was identified and shown to have convincing efficacy in *in-vivo* models of TB when delivered orally[19,20]. As indicated by recent reports[21] of WHO, there is a resurgence of TB which is one of the primary infectious diseases worldwide , even in civilized countries in Europe and North America due to its pathogenic synergy with HIV. New drugs are critically needed to combat this disease. In fact, the battle against tuberculosis which seemed to be over by 1985 is back and the discovery of effective new antitubercular drugs must surely be one of the most urgent priorities. This organism was proven to be very resilient and a tough adversary because of the emergence of multiple-drug-resistant TB organisms, a term used to describe strains that are resistant to two or more of the five first-line anti-TB drugs (isoniazide, ethambutol, rifampicin, pyrizinamide and streptomycin)[22].

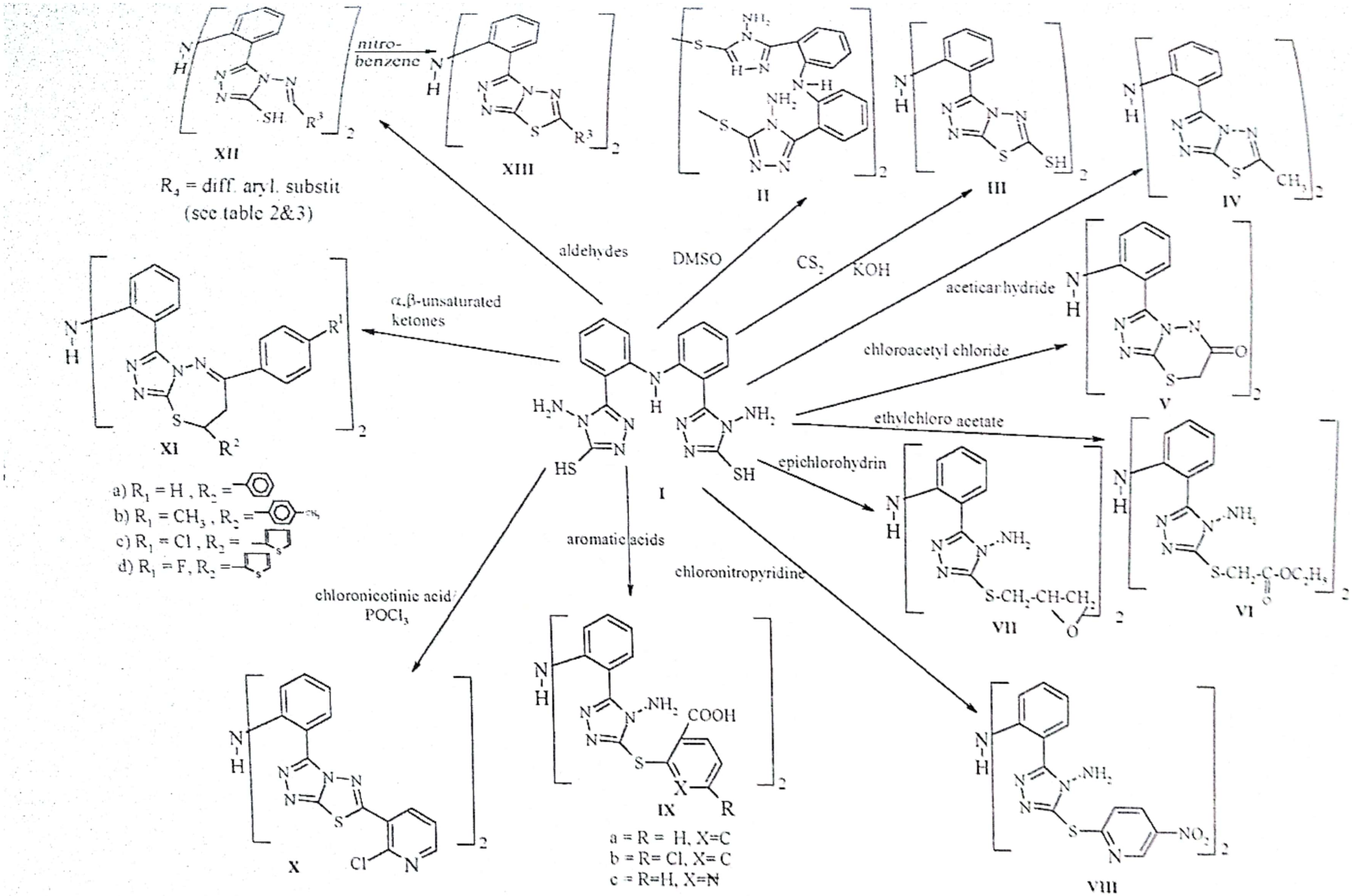
Different heterocycle-containing compounds proved to exhibit anti-TB activity[23,24]. Some triazole-containing compounds were of special interest as they showed variation in activities according to the structure in which it was incorporated[25-28]. In lieu of continuing the investigations for the possible activities attributed to triazoles, it deemed of interest to synthesise some new bis-triazole derivatives (see Scheme 1). This was achieved through further substitution and/or extension of the starting intermediate (**I**). Some of these new derivatives were tested for their antitubercular activity.

CHEMISTRY

The starting s-triazole key intermediate (**I**) was prepared as reported[29] under specific reaction conditions, via carbon disulphide addition to an ethanolic solution of diphenylamine-2,2`-dicarboxylic acid hydrazide, containing potassium hydroxide, followed by the subsequent addition of hydrazine hydrate. This compound (**I**), features a number of functional groups and the usual tactics were applied through substitution on these groups and formation of newly added ring systems in an attempt to get compounds with better activity. Thus, two molecules of **I** were linked through the sulphur atom, when it was refluxed in DMSO[30,31] to give **II**. When this s-triazole derivative (**I**) was heated under reflux with carbon disulphide and potassium hydroxide[32-34], further cyclisation including both the amino and thiol groups took place giving an extra five-membered ring and the corresponding bis-mercaptotriazolothiadiazole compound (**III**) was obtained. Attempted cyclisation of **I** using acetic anhydride[34] was successful giving the bis-methyltriazolothiadiazole derivative (**IV**). On the other hand, cyclisation using chloroacetyl chloride furnished the bis-triazolothiadiazine analogue (**V**). Reaction of the two thiol groups of **I** with ethyl chloroacetate[35,36], epichlorohydrin[36,37] or 2-chloro-5-nitropyridine afforded the corresponding thioethers (**VI-VIII**) respectively . Consequently, reacting **I** with different o-chloro substituted aromatic acids[31], resulted in **IX**. Furthermore, when the reaction was carried out with o-chloronicotinic acid in $POCl_3$[32,38], it gave **X** (Scheme1).

The reaction between **I** and different α, β-unsaturated ketones[31,39] through Michael addition reaction furnished compounds having a triazolothiadiazepine moiety (**XI**),where both the amino and thiol groups of (**I**) were involved (Scheme1). The ^{13}C-NMR of **XIa** showed the presence of a peak at δ190 ppm indicating C=N formation.

Increasing the temperature of the reaction leading to **I** above the reported 40°C, led to an unambiguous product, 2-hydrazino carbonyl-2`-(4-amino-5-mercapto-1,2,4-triazol-3-yl)diphenylamine (**A**), which when reacted with an ά,β-unsaturated ketone gave 2-{6,8-bis(p-tolyl)7,8-dihydro-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazepin-3-yl}–2`-{3,5-bis(p-tolyl)-1,4-dihydropyridazin-6-yl}diphenylamine (**B**) (Scheme 2). The structure of compound **B** was proven by microanalysis, and mass spectrum. Schiff bases (**XII**) were obtained via reaction of **I** and various aldehydes[32,34,40]. Finally, further thermal induced cyclisation of **XII** was carried out by reflux in nitrobenzene[40] to give **XIII**. (Scheme 1)



Scheme 1

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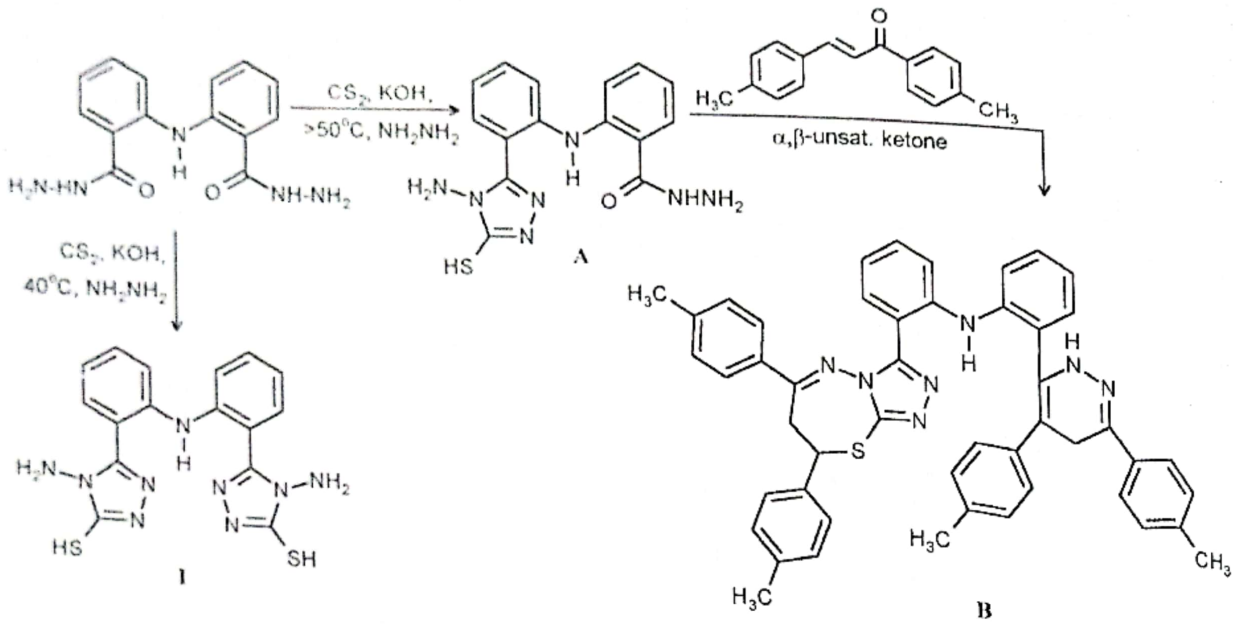

Scheme 2

EXPERIMENTAL

Melting points (°C, uncorrected) were recorded on an Electrothermal I A 9100 Digital Melting Point Apparatus. ^{1}H NMR spectra were recorded in DMSO-D_6 on a Mercury, Varian, Oxford, 300 MHz & Jeol FX 90 Q 90 MHz Fourier Transform NMR spectrometer, using TMS as internal standard (chemical shifts in δ ppm) . Micro analytical data (C, H, N, S &/or halogen) agreed with the proposed structures within the approved ranges. Elemental analyses were carried out at the Micro analytical Center, Cairo University and few at Micro Analytical Lab., Chemistry Department, Faculty Of Science, Ain Shams University. U.V. spectra were carried out in ethanol and recorded on a Schimadzu UV 265 spectrophotometer. IR spectra as KBr pellets were recorded on a Schimadzu 435 IR –spectrophotometer. The mass spectra were recorded on a HP-Model –MS –5800.

Dimer of bis-[2-(4-amino-5-thio[1,2,4]triazol-3-yl) phenyl]amine (II):

A solution of **I** (0.4g, 0.001 mol) in dimethyl sulphoxide (5 ml), was heated under reflux for an hour. The solution was cooled and poured onto crushed-ice with stirring. The buff precipitate produced was filtered, left to dry to yield 0.33 g (83%) (crude), (a TLC was performed of (**II**) against (**I**) using ethanol : chloroform in a ratio of 5:1), which was crystallized from aqueous ethanol , mp = 153-4°C IR: νcm^{-1}: 3250, 3150 ($NH_{2(s)}$, $NH_{(s)}$), 1610, 1580, 1500 (NH, C = N , C=C). U.V.: λ max (log ε): 334.2 (4.3), 285 (sh) (4.56), 255 (4.90). MS: m/z = 791.6 (M^+, 100 %) , Calc. = 790.9.

Calculated for $C_{32}H_{26}N_{18}S_4$: C, 48.6; H, 3.3; N, 31.9. Found: C, 49.0; H, 3.5; N, 31.5

Bis-[2-(6-mercapto[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazol-3-yl)phenyl] amine (III):

Compound **I** (0.5 g, 0.0013 mol) was dissolved in a solution of potassium hydroxide (0.22 g) in absolute ethanol (5 ml), carbon disulphide (1 ml) was then added portionwise with stirring . The reaction mixture was heated under reflux for 6 hrs. and the solvent evaporated . To the remaining residue, 5ml water was added and the reaction mixture was acidified with 10% HCl till pH =1. The gelatinous precipitate produced was filtered, washed well with water, dried to give 0.6g (99%) which was recrystallised from DMF/H_2O , mp = 203-4°C . U.V.: λmax (log ε): 355 (sh) (3.98) , 344 (4.02) , 285 (sh) (4.35) , 255 (4.68) . ^{1}H NMR δ ppm = 5.50 (s (br.), 2H, 2SH), 7.5-8.8 (m, 2×4H, arom. protons), 13.2 (m, 1H, NH, exchangeable).

Calculated for $C_{18}H_{11}N_9S_4$: C, 44.9; H, 2.3; N, 26.2. Found: C, 44.5; H, 2.6; N, 26.0

Bis-[2-(6-methyl[1,2,4]triazolo[3,4-b]-[1,3,4]thiadiazol-3-yl)phenyl] amine (IV) :

A solution of **I** (0.3 g, 0.00075 mol) was heated under reflux in acetic anhydride (5ml) for 5 hrs. The reaction mixture was cooled and neutralized with dil. ammonium hydroxide solution. The precipitate formed was collected and dried to give 0.2 g (60%).

The solid was crystallized from DMF/H_2O, mp = 225-6°C. MS: m/z = 445.5 (M^+, 17%).

Calculated for $C_{20}H_{15}N_9S_2$: C, 53.9; H, 3.4

Found: C, 53.7; H, 3.7

Bis-[2-(6-oxo-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b]-[1,3,4]thiadiazin-3-yl) phenyl]amine (V) :

To a solution of **I** (0.35 g, 0.0009 mol) in absolute ethanol (5 ml), chloroacetyl chloride (0.22ml) was added and heated under reflux for 10 hrs . The solvent was concentrated then poured over ice. The precipitate formed was filtered and dried to yield 0.31g (74%). The obtained solid was recrystallized from aqueous ethanol, mp = 113-5°C. IR : νcm^{-1}: 3300 ($NH_{(s)}$), 2900 ($CH_{2(s)}$), 1730 ($CO_{(s)}$) , 1620, 1590 (NH, C=N, C=C). U.V: λmax (logε): 329.8 (4.08) , 285.0 (sh)(4.27), 255 (4.67), 251.4 (4.68) .

Calculated for$C_{20}H_{15}N_9O_2S_2$: C, 50.3; H, 3.2; N, 26.4. Found: C, 50.4; H, 3.5; N 26.2

Bis-[2(4-amino-5-ethoxycarbonylmethylthio-[1,2,4]triazol-3-yl)phenyl] amine (VI):

A mixture of **I** (0.5 g , 0.0013 mol), ethyl chloroacetate (2.0 ml, 0.016 mol), anhydrous potassium carbonate (0.75 g) in dry acetone (120 ml) was heated under reflux for 11 hrs. with stirring , filtered , concentrated , cooled and poured over crushed ice . The oily layer produced was separated and crystallized from acetone to give 0.67g (93%), mp = 91-3°C. IR: νcm^{-1} : 3300 (br) ($NH_{2(s)}$, NH), 2970 , 2900 ($CH_{2(s)}$, $CH_{3(s)}$), 1740 ($CO_{(s)}$) , 1620 , 1590, 1540 (NH, C=N, C=C). U.V.: λ max (logε): 333.0 (3.90), 295.0 (sh) (4.01), 260.0 (sh) (4.29), 242.8 (4.93). MS: m/z = 568.4 (M-1,17%), calc. = 569.7 .

Calculated for $C_{24}H_{27}N_9O_4S_2$: C, 50.6; H, 4.8; N, 22.1; S, 11.3. Found: C, 50.6; H, 4.7; N, 22.5; S, 11.4

Bis-[2-(4-amino-5-(2-oxiranylmethylthio)[1,2,4]-triazol-3-yl)phenyl amine (VII):

Compound **I** (0.31 g, 0.008 mol) and epichlorohydrin (0.45 g) in dry absolute ethanol (30 ml) were refluxed for 7 hrs. . The reaction mixture was concentrated, left in the fridge for several days to allow complete precipitation. The solid obtained was collected, left to dry to give 0.16g (41%). It was recrystallized from DMF/H_2O, mp = >350°C. IR: νcm^{-1} : 3350 ($NH_{2(s)}$, NH) , 2970 , 2900 ($CH_{2(s)}$, $CH_{(s)}$), 1600, 1550, 1500 (NH, C=N, C=C). U.V.: λ max (logε): 335.0 (3.94), 290.0 (sh) (4.11), 242.8 (4.88). ^1H NMR: δ ppm = 1.10 (d, 4H, 2 × CH_2 oxiranyl) , 2.67 (m, 2H, CH oxiranyl), 3.90 (d, 4H , S-CH_2) , 5.50 (s(br) , 4H , 2 x NH_2 exchangeable), 6.88 – 8 (m, 8H , arom. protons) , 9.1 (s ,1H , NH exchangeable) .

Calculated for $C_{22}H_{23}N_9O_2S_2$: C, 51.9; H, 4.6; N, 24.7. Found: C, 52.2; H, 4.9; N, 24.5

Bis-[2-(4-amino-5-(5-nitropyrid-2-ylthio)-[1,2,4]-triazol-3-yl)phenyl] amine (VIII):

A mixture of **I** (0.4g , 0.001mol) , 2-chloro-5-nitropyridine (0.32 g, 0.002 mol), sodium acetate anhydrous (0.21g) in absolute ethanol (25ml) was heated under reflux for 12 hrs., it was then concentrated , cooled , the precipitate collected and dried to give 0.63g (97%). The precipitate was recrystallized from ethanol with a mp = 166-8°C. IR: νcm^{-1}: 3450-3350 (br) ($NH_{2(s)}$, NH), 1600, 1580 (NH, C=N, C=C), 1520,1355 (NO_2). U.V.: λ max (log ε): 303.8 (4.46) , 295.0 (sh)(4.44) , 251.8 (4.42) , 239.0 (4.20). ^1H NMR : δppm = 6.95(s, 2×2H , $2NH_2$ exchangeable), 7.17-10.22 (m, 15H, arom.protons +NH exchangeable)

Calculated for $C_{26}H_{19}N_{13}O_4S_2$: C, 48.7; H, 3.0; N, 28.4. Found: C, 49.1; H, 3.2; N, 28.1

Bis-[2-(4-amino-5-arylthio[1,2,4]triazol-3-yl)-phenyl]amine (IXa-c):

A mixture of **I** (0.4 g , 0.001 mol) , the appropriate chloro substituted aromatic acid (0.002 mol), anhydrous sodium acetate (0.21 g) in absolute ethanol (25 ml) , was heated under reflux for 3-15 hrs. The solution obtained was concentrated, cooled, precipitated by ice, filtered , left to dry and recrystallised from aqueous ethanol. IR: νcm^{-1} (IXa): 3500-2700 (broad overlapped bands) (OH, NH_2, NH), 1680, 1620, 1590, 1475 (C=O, C=C, C=N). **(IXb)**: 3500-2800 (broad overlapped bands) (OH, NH_2, NH), 1670, 1620, 1590, 1470 (C=O, C=C, C=N). **(IXc)**: 3500-2800 (broad overlapped bands) (OH , NH_2 , NH), 1670, 1630, 1590, 1480 (C=O, C=C, C=N). MS for **(IXb)**: calculated for $C_{30}H_{21}Cl_2N_9O_4S_2$= 706.6, m/z=706.2($M^+$, 84%).(See Table 1).

Bis-[2-(6-(2-pyridyl)[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazol-3-yl)phenyl] amine (X):

A mixture of **I** (0.4 g, 0.001 mol), 2-chloro-nicotinic acid (0.32 g, 0.002 mol), was heated under reflux in $POCl_3$ (10 ml) for 5 hrs. The excess $POCl_3$ was distilled under vacuum and the remaining oily residue was cautiously poured over crushed ice with stirring and scratching to get a brown solid. The reaction mixture in hand was alkalinized with a 10% aqueous sodium hydroxide solution till pH = 5. The separated solid was collected, washed well with water and dried to give 0.6g (94 %) of the compound which was then crystallized from DMF/ H_2O, mp = 255-7°C. U.V.: λ max (log ε): 290.0 (sh)(4.31), 242.8 (5.26). MS : m/z = 639.55 (M-1), calc.= 640.55 .

Calculated for $C_{28}H_{15}Cl_2N_{11}S_2$: C, 52.5; H, 2.4. Found: C, 52.5; H, 2.4

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Table 1: Physical and analytical data of compounds IX_{a-c} and XI_{a-d}

Cpd No	Yield %	Molecular Formula	Mol. Wt.	m.p.(°C) Cryst.solvent	U.V. λmax(logε)	Microanalysis Calc.	Found
IX a	58	$C_{30}H_{23}N_9O_4S_2$	637.703	170-2 Ethanol/H_2O	333.2 (4.29), 280 (sh) (4.55), 250.4 (4.92), 245.0 (sh)(4.90).	C 56.5 H 3.6 N 19.8	56.5 3.6 20.0
b	31	$C_{30}H_{21}Cl_2N_9O_4S_2$	706.601	169-71 Ethanol/H_2O	334.6 (4.22), 280(sh) (4.49), 250 (sh) (4.85), 242.2 (4.97).	C 51.0 H 3.0 N 17.8	51.0 3.1 17.7
c	54	$C_{28}H_{21}N_{11}O_4S_2$	639.678	190-2 Ethanol/H_2O	333.6 (4.29), 280(sh) (4.56), 250.4 (4.90).	C 52.6 H 3.3 N 24.1	52.6 3.3 24.3
XI a	96	$C_{46}H_{33}N_9S_2$	777.978	68-70 Ethanol/H_2O	312.0 (sh)(4.88), 305.0 (4.89), 301.8 (4.89), 295.0 (4.88), 290.0 (4.82), 265.2 (4.71), 256.6 (4.74), 250 (4.73).	C 71.0 H 4.5 N 16.2	70.9 4.3 16.2
b	96	$C_{50}H_{43}N_9S_2$	834.086	97-8 Ethanol/H_2O	318.6 (4.79), 258.0 (sh)(4.77), 243.4 (5.22).	C 72.0 H 5.2 N 15.1	72.2 5.3 15.0
c	90	$C_{42}H_{29}Cl_2N_9S_4$	858.928	111-3 Ethanol/H_2O	346.4 (5.12), 277.4 (4.99), 270.8 (4.99), 257.0 (sh)(4.97), 239.6 (4.86).	C 58.7 H 3.4 N 14.7	59.0 3.5 14.6
d	89	$C_{42}H_{29}F_2N_9S_4$	826.010	82-3 Ethanol/H_2O		C 61.1 H 3.5 N 15.3	61.0 3.6 15.5

Table 2: Physical and Analytical data of compounds XII_{a-i}

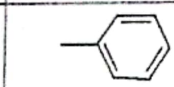
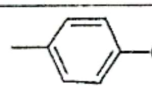
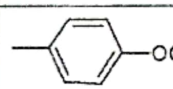
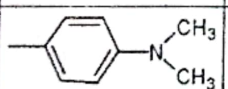
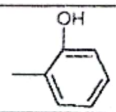
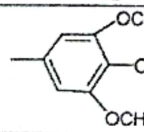
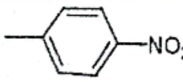
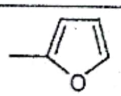
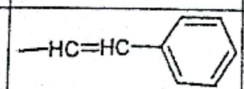
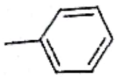
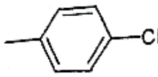
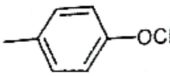
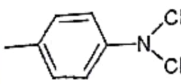
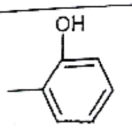
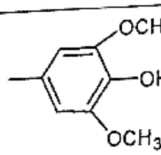
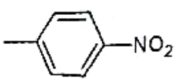
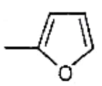
Cpd No	R^3	Yield %	Molecular Formula	Mol. Wt.	m.p.(°C) Cryst. solvent	U.V. λmax(logε)	Microanalysis Calc.	Found
XIIa		57	$C_{30}H_{23}N_9S_2$	573.705	249-50 DMF/H_2O	328.0 (4.26), 256.4 (4.83), 235.4 (4.37).	C 62.8 H 4.0 N 22.0	62.5 3.8 21.6
b		96	$C_{30}H_{21}Cl_2N_9S_2$	642.603	165-6 Ethanol/DMF/H_2O	330.0 (sh)(4.23), 280.0 (4.81), 257.6 (4.81), 238.0 (4.24).	C 56.1 H 3.3 N 19.6	56.3 3.5 19.4
c		91	$C_{32}H_{27}N_9O_2S_2$	633.757	167-9 Ethanol/H_2O	340.0 (sh)(4.44), 291.6 (4.85), 253.6 (4.80).	C 60.7 H 4.3 N 19.9	60.7 4.4 19.6
d		92	$C_{34}H_{33}N_{11}S_2.H_2O$	677.857	214-6 DMF/H_2O	358.0 (4.63), 305.0 (sh)(4.24), 253.6 (4.37).	C 60.3 H 5.2 N 22.7	60.3 4.7 22.6
e		95	$C_{30}H_{23}N_9O_2S_2$	605.704	169-70 DMF/Ethanol/H_2O	335.6 (4.52), 295.0 (sh)(4.51), 255.4 (4.91).	C 59.5 H 3.8 N 20.8	59.2 4.1 20.5
f		98	$C_{34}H_{31}N_9O_6S_2.H_2O$	743.825	192-4 DMF/H_2O	324.4 (4.98), 255.0 (sh)(4.91), 245.8 (5.01).	C 54.9 H 4.4 N 16.9	54.5 4.4 17.3
g		96	$C_{30}H_{21}N_{11}O_4S_2$	663.700	185-6 DMF/Ethanol/H_2O	380.0 (sh)(4.50), 286.2 (5.16), 242.8 (5.42).	C 54.3 H 3.2 N 23.2	54.1 3.6 23.0
h		95	$C_{26}H_{19}N_9O_2S_2$	553.627	157-9 DMF/Ethanol/H_2O	320.0 (sh)(4.62), 288.4 (4.95), 252.0 (sh) (4.95), 243.0 (5.04).	C 56.4 H 3.5 N 22.8	56.5 3.7 23.0
I		99	$C_{34}H_{27}N_9S_2$	625.781	198-200 DMF/H_2O	299.2 (4.96), 257.0 (sh)(4.88), 242.0 (5.05).	C 65.3 H 4.4 N 20.1	65.2 4.7 19.9

Table 3: Physical and analytical data of compounds **XIII$_{a-h}$**

Cpd No	R^3	Yield %	Molecular Formula	Mol. Wt.	m.p.(°C) Cryst. solvent	U.V. λmax(logε)	Microanalysis Calc.	Found
XIIIa		80	$C_{30}H_{19}N_9S_2$	569.673	114-6 DMF/H_2O	355.3 (4.21), 308.1 (4.39), 272.5 (4.66), 257.0 (4.59), 240.0 (4.49).	C 63.3 H 3.4 N 22.1 S 11.3	63.3 3.3 21.9 11.2
b		98	$C_{30}H_{17}Cl_2N_9S_2$	638.571	283-5 DMF/H_2O	551.0 (3.94), 372.0 (sh)(4.25), 359.6 (sh)(4.37), 309.7 (4.67), 271.3 (4.75), 257.0 (4.75), 243.0 (4.75).	C 56.4 H 2.7 N 19.7 Cl 11.1	56.4 2.6 19.4 11.2
c		98	$C_{32}H_{23}N_9O_2S_2$	629.726	184-6 DMF/H_2O	558.5 (3.85), 366.8 (sh)(4.26), 359.6 (sh)(4.43), 314.0 (4.74), 257.0 (4.74), 238.0 (4.74).	C 61.0 H 3.7 N 20.0 S 10.2	61.0 3.7 19.6 10.3
d		95	$C_{34}H_{29}N_{11}S_2$	655.81	255-7 DMF/H_2O	551.5 (3.77), 362.0 (sh) (4.19), 346.8 (4.37), 292.6 (sh) (4.49), 255.6 (4.70).	C 62.3 H 4.5 N 23.5	62.5 4.4 23.1
e		72	$C_{30}H_{19}N_9O_2S_2$	601.672	208-10 DMF/Ethanol/ H_2O	363.0 (sh)(4.18), 359.2 (4.33), 309.7 (4.57), 266.0 (4.75), 255.0 (4.70).	C 59.9 H 3.2 N 21.0 S 10.7	59.0 3.2 20.6 10.6
f		98	$C_{34}H_{27}N_9O_6S_2$	721.778	242-4 Ethanol/DMF/ H_2O	545.0 (3.28), 365.0 (sh)(4.10), 356.8 (4.24), 306.5 (4.48), 264.1 (4.71), 257.0 (4.75), 245.0 (4.65).	C 56.6 H 3.8 N 17.5 S 8.9	56.6 3.7 17.6 9.0
g		93	$C_{30}H_{17}N_{11}O_4S_2$	659.669	223-5 Ethanol/DMF/ H_2O	560.5 (4.20), 363.9 (sh)(4.23), 358.2 (sh)(4.39), 312.6 (4.77), 268.4 (4.75), 257.0 (4.76), 238.0 (4.76).	C 54.6 H 2.6 N 23.4 S 9.7	54.7 2.6 23.1 9.8
h		97	$C_{26}H_{15}N_9O_2S_2$	549.596	More than 350 (278 in a sealed tube) Ethanol/DMF/ H_2O	453.7 (3.92), 363.0 (sh)(4.16), 358.2 (sh)(4.26), 271.3 (4.55), 257.0 (4.63), 242.0 (4.54).	C 56.8 H 2.8 N 22.9	56.7 2.9 22.9

Bis[2-(6,8-disubstituted[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazepin-3yl)phenyl]amine (XIa-d):

A mixture of compound **I** and the appropriate α, β-unsaturated ketone in a ratio of 1:2 was heated under reflux for 9-14 hrs. in the least amount of acetic acid . The mixture was left to cool, precipitated on crushed ice, the precipitate was collected, dried and crystallized from aqueous ethanol. (see Table 1). IR: νcm^{-1}: (**XIb**): 3350 (br)(NH), 2950, 2800 ($CH_{3(s)}$, $CH_{2(s)}$), 1665 (C=N of thiadiazepine ring), 1605, 1575, 1520, 1500 (NH, C= N , C=C).

^{1}H-NMR (**XIa**) δppm: 2.51(d , 2 × 2H ,CH_2) , 5.7-5.85(m,2 x 1H, S-CH), 7.06-8.15 (m, 2×14H, aromatic), 9.57 (s (br), 1H, NH, exchangeable). (**XIc**) δppm: 2.51(d, 2x2H, CH_2), 5.8-6.05 (m, 2x1H, S-CH), 7.16-8.25 (m, 2×11H, aromatic), 9.59 (s (br), 1H, NH, exchangeable). ^{13}C-NMR (**XIa**) (DMSO-d_6): 39.45, 41.2, 122.92, 129.17, 129.45, 129.51, 129.59, 131.28, 133.37, 133.76, 135.37, 138.32, 144.67, 190.00. MS: for (**XIc**)= (calculated for $C_{42}H_{29}Cl_2N_9S_4$ = 858.93), m/z = 858.45 (M^+)

Bis-[2-(5-mercapto-4-arylideneamino-[1,2,4]-triazol-3-yl) phenyl]amine (XIIa-i):

The appropriate aldehyde (0.0028 mol) was added to a hot solution of **I** (0.0013 mol) in acetic acid (7 ml). The solution was refluxed for 2-8 hrs., concentrated, the precipitate filtered, allowed to dry then crystallized from the appropriate solvent. (See

Table 2). IR νcm^{-1}: **(XIIa)**: 3382 (NH), 2774 (SH) , 1604,1576,1550(NH, C = N , C=C).**(XIIe)**: 3600 (OH), 3450 (NH), 1630,1615, 1600(NH, C=N , C=C).

Bis[2-(6-aryl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl) phenyl] amine (XIIIa-h):

The Schiff bases **XII** were heated under reflux in nitrobenzene with stirring for 2-3 hrs. Excess nitrobenzene was distilled under vacuum till dryness with the aid of ethanol. The dried residue was crystallized from the suitable solvent. (See Table 3) IR νcm^{-1}: **(XIIIa)**: 3300 (NH), 1658, 1587, 1500(NH, C=N , C=C). **(XIIIf)**: 3400-3250 (br)(OH, NH), 2950 (CH_{Ar}), 1595, 1570 (NH, C=N , C=C). MS: for **(XIIIa)**: (calculated for $C_{30}H_{19}N_9S_2$ = 569.67), m/z = 571 (M'+2). MS: for **(XIIIh)**: (calculated for $C_{26}H_{15}N_8O_2S_2$ = 549.60), m/z = 551.95 (M'+2, 7%).

Antitubercular Activity :

Evaluation of antimycobacterial activity -*in vitro*- was carried out through a primary screening conducted at 6.25 µg/ml (or molar equivalent of the highest molecular weight compound in a series of congeners) against Mycobacterium tuberculosis H_{37} Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA)[41-43]. The Alamar blue oxidation-reduction dye is a general indicator of cellular growth and/or viability; the blue, nonfluorescent, oxidized form becomes pink and fluorescent upon reduction. Growth can therefore be measured with a fluorometer or spectrophotometer or determined by a visual color change.

Antimicrobial susceptibility testing was performed in black, clear-bottomed, 96-well microplates. Initial drug dilutions were prepared in either dimethyl sulphoxide or distilled deionised water and subsequent twofold dilutions were performed in 0.1ml of 7H9GC (no Tween 80) in the microplates. BACTEC 12B inocula were initially diluted 1:2 in 7H9GC and 0.1ml was added to the wells. Wells containing drug only were used to detect autoflourescence of compounds. Additional control wells consisted of bacteria only (B) and medium only (M). Plates were incubated at 37°C. Starting at day 4 of incubation, 20µl of alamar blue solution and 12.5 ml of 20% Tween 80 were added to one B well and one M well and plates were reincubated at 37°C. Wells were observed at 12 and 24 hrs for color change from blue to pink and the fluorescence measured. Compounds demonstrating about 90% inhibition in the primary screen were retested at lower concentrations against M. tuberculosis H_{37}Rv to determine the actual minimum inhibitory concentration (MIC) using MABA (Table 4).

The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Concurrent with the determination of MICs, compounds were tested for cytotoxicity (IC50) in VERO cells at concentrations < 6.25 mg /ml or 10×the MIC for M. tuberculosis H37Rv (solubility in media permitting). After 72 hours exposure, viability is assessed on the basis of cellular conversion of MIT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell Proliferation Assay.

Table 4: Antitubercular Activity

Compd. No	MIC(ug/ml)	% Inh.
I	> 6.25	-18
II	> 6.25	10
III	> 6.25	-39
V	> 6.25	22
VIII	> 6.25	-23
XI a	> 6.25	79
c	< 6.25	94
XII a	> 6.25	-40
b	> 6.25	-40
c	> 6.25	-47
d	> 6.25	-75
e	> 6.25	-58
f	> 6.25	4
g	> 6.25	16
h	> 6.25	-30
I	> 6.25	-8
XIII a	> 6.25	11
b	> 6.25	83
c	> 6.25	16
d	> 6.25	16
e	> 6.25	16
f	> 6.25	32
g	> 6.25	5
h	> 6.25	31

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تشييد واختبار الفعالية ضد مرض الدرن لمشتقات ١،٢،٤-الترايازول

أمل عبد الحليم عيسى

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يعتبر مرض الدرن من الأمراض الانتهازية المنتشرة و الإصابة به في ازدياد في مرضى نقص المناعة المكتسبة بالتحديد وبالتالي فالاحتياج الى مركبات جديدة لعلاجه مطلوب دائما. لهذا تم في هذا البحث تحضير عدد من المركبات التي تحتوي على حلقتين من حلقات الترايازول باستخدام مجموعتي الأمينو والثيول الموجودتين في المركب ٢،٢-ثنائي (٤-امينو-٥-مركبتو-٤هـ-١،٢،٤-ترايازول-٣-ايل) ثنائي فينيل امين (I). تفاعل (I) في ثنائي ميثيل سلفوكسايد، مع ثاني كبريتيد الكربون فى هيدروكسيد البوتاسيوم، الهيدرازيد حمض الخليك، كلوريد الكلورو استيل، كلورو خلات الايثيل، الايبي كلورو هيدرين، ٢-كلورو-٥-نيترو بيريدين، اريل كلورو بعض الاحماض الاروماتية (أو في وجود اوكسي كلوريد الفوسفور)، الكيتونات غير المشبعة في الوضع الفا-بيتا، والدهيدات مختلفة لإعطاء المركبات II-XII. وقد تم حلقنة المركبات الأخيرة (XII) بواسطة التسخين في النيتروبنزين لإعطاء المركبات رقم XIII.

معظم المركبات تم الكشف عن فاعليتها ضد مرض الدرن. وقد وجد أن المركبات رقم XIIIb, XIc لها نشاط ضد الميكروب المستخدم.