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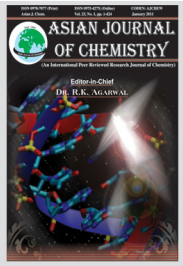

# Synthesis and Antimicrobial Activity of 1,2,4-Triazolo[4,3-b][1,2,4,5]tetrazines

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Synthesis of 4-phenylamino-5-phenyl-4*H*-[1,2,4]triazole-3-thiol (**1**) and 3-methylthio-4-phenylamino-5-phenyl-4*H*-[1,2,4]triazole (**2**) is described. Reactions of hydrazonoyl halides (**3**) with either **1** or **2** afforded 1,2,4-triazol[4,3-b][1,2,4,5]tetrazine. The latter products were screened for their antifungal and antibacterial properties. The structure of the products was established based on elemental and spectral analyses. Further evidence for the assigned structure of the products is based on alternative synthesis. The mechanism of the studied reactions was also discussed.

**Key Words: Hydrazonoyl halides, Nitrilimines, Thiohydrazonate esters, 1,2,4-Triazolo[4,3-b][1,2,4,5]tetrazines.**

## INTRODUCTION

Organic compounds with high-nitrogen content currently attracted significant attention of many researchers to their novel energetic properties[1-3]. Moreover, many derivatives of 1,2,4,5-tetrazine ring system have also attracted considerable attention of many research groups because of their diverse and interesting biological activity[4-7], as well as antiviral and antitumour activities[8-11]. On view of these findings, we wish to report herein a facile synthesis of 1,2,4-triazolo[4,3-b][1,2,4,5]tetrazines *via* reactions of hydrazonoyl halides, with 4-pheylamino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**1**) and its 3-methyl thio derivative (**2**). In addition, some of the newly synthesized compounds were screened for their antibacterial and antifungal activities.

## EXPERIMENTAL

All melting points were determined on an electrothermal Gallenkamp apparatus and are uncorrected. Solvents were generally distilled and dried by standard literature procedure prior to use. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The $^1$H NMR spectra were recorded on a Varian Mercury VXR-300 spectrometer (300 MHz). The mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt.

Hydrazonoyl halides (**3**) were prepared by literature methods[12,13].

**Synthesis of 4-phenylamino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (1):** To an ethanolic potassium hydroxide solution, prepared from potassium hydroxide (8.4 g, 0.15 mol) and absolute ethanol (100 mL), was mixed with benzoic acid hydrazide (13.6 g, 0.1 mol) until the solution became clear. To the clear solution was added carbon disulfide (0.15 mol). The solution was stirred for 3 h at 25 ºC and then ethyl ether 100 mL was added to form a precipitate. The precipitate was filtered and washed with ethyl ether several times. The precipitate was mixed with 160 mmoles of phenyl hydrazine and 2 mL of water. The solution was refluxed for 2 h until the colour of the solution became clear green. After cooling to room temperature, 100 mL of ice water was added to the solution and neutralized with 3 N HCl to form a precipitate. The precipitate was isolated by filtration and crystallized from ethanol to give pure compound **1**. White crystals (17.44 g, 65 %), m.p. 223 ºC, (EtOH) (Lit.[14] 229 ºC); IR (KBr, $\nu_{max}$, cm$^{-1}$): 3240, 3180, 2926, 1600; $^1$H NMR ($CDCl_3$) δ: 6.70-7.98 (m, 10H, ArH'S), 9.97 (s, 1H, NH), 13.56 (s, 1H, SH); MS m/z (%) 269 (16), 268 (85), 235(42), 104 (57), 92 (100), 51 (64); Anal. calcd. for $C_{14}H_{12}N_4S$ (268.34): C, 62.66; H, 4.51; N, 20.88. Found: C, 62.64; H, 4.49; N, 20.85 %.

**3-(Methylthio)-N,5-diphenyl-4*H*-1,2,4-triazol-4-amine (2):** To an ethanolic sodium ethoxide solution, prepared from sodium metal (0.23 g, 0.01 mol) and absolute ethanol (100 mL), was added compound **1** (2.68 g, 0.01 mol) with constant stirring. To the resulting solution was added methyl iodide (1.42 g, 0.01 mol) and the mixture was refluxed on a water bath for 1 h, then left at room temperature overnight. The

precipitated solid was collected by filtration and crystallized from ethanol to give pure **2** as white crystals (0.99 g, 35 %), m.p. 214 ºC (EtOH), (Lit.[15] 218-220 ºC); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3280, 3200, 2950, 1602; $^1H$ NMR ($CDCl_3$) δ: 2.65 (s, 3H), 6.78-8.08 (m, 10H, ArH'S), 9.84 (s, 1H, NH); MS m/z (%) 283 (12), 282 (25), 234 (35), 103 (36), 92 (100), 52 (52); Anal. calcd. for $C_{15}H_{14}N_4S$ (282.37): C, 63.81; H, 5.00; N, 19.84. Found: C, 63.79; H, 4.92; N, 19.81 %.

**Prepration of thiohydrazonates (5a-f):** To an ethanolic sodium ethoxide solution, prepared from sodium metal (0.11 g, 5 mmol) and absolute ethanol (20 mL) was added the triazolethione (**2**) (1.34 g, 5 mmol). After stirring the mixture for 15 min, the appropriate hydrazonoyl halide (**3**) (5 mmol) was added and stirring continued for 12 h. During this period, the hydrazonoyl halide dissolved and new precipitate was formed. The reaction mixture was then left overnight at room temperature. The crude product that precipitated was filtered off, washed with water and finally crystallized from the appropriate solvent to give the respective thiohydrazonate ester (**5**). The compounds **5a-f** prepared, together with their physical constants, are listed below.

Reactions of **2** with each of **3g-i**, when carried out as above, afforded directly the triazolotetrazine derivatives (**7g-i**), respectively.

**4-Phenylamino-5-phenyl-1,2,4-triazol-3-yl-N-phenyl-2-oxopropane thiohydrazonote (5a):** Pale yellow solid (1.61 g, 75 %), m.p. 232 ºC (dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3142, 1678, 1640; $^1H$ NMR (DMSO-$d_6$) δ: 2.48 (s, 3H), 6.71-8.65 (m, 15H), 10.35 (s, 2H, $D_2O$ exchangeable); MS m/z (%) 428 ($M^+$, 39), 412 (31), 382 (25), 351(23), 313 (68), 136 (48), 95 (50), 76 (100), 59 (58), 51 (51); Anal. calcd. for $C_{23}H_{20}N_6OS$ (428.52): C, 64.47; H, 4.70; N, 19.61. Found: C, 64.41; H, 4.68; N, 19. 58 %.

**4-Phenylamino-5-phenyl-1,2,4-triazol-3-yl-N-(4-tolyl)-2-oxopropane thiohydrazonote (5b):** Yellow solid (1.77 g, 80 %); m.p. 190-192 ºC (EtOH/DMF); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3150, 1675, 1645; $^1H$ NMR (DMSO-$d_6$) δ: 2.07 (s, 3H), 2.46 (s, 3H), 6.69-8.17 (m, 14H), 10.46 (s, 2H, $D_2O$ exchangeable); MS m/z (%) 442 ($M^+$, 25), 352 (37), 197 (13), 132 (44), 105 (100), 91 (93), 76 (58), 51(20); Anal. calcd. for $C_{24}H_{22}N_6OS$ (442.55): C, 65.14; H, 5.01; N, 18.99. Found: C, 65.11; H, 5.00; N, 18.89 %.

**4-Phenylamino-5-phenyl-1,2,4-triazol-3-yl-N-(4-chlorophenyl)-2-oxopropane thiohydrazonote (5c):** Yellow solid (1.73 g, 75 %); m.p. 200-202 ºC (EtOH/DMF); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3191, 3112, 1685, 1640; $^1H$ NMR (DMSO-$d_6$) δ: 2.50 (s, 3H), 7.38-8.17 (m, 14H), 11.23 (s, 2H, $D_2O$ exchangeable); MS m/z (%) 464 ($M^+$+2, 71), 462 ($M^+$, 65), 374 (62), 332 (48), 315 (62), 263 (95)214 (48), 162 (57), 135 (48), 105 (86), 82 (100), 73 (57), 62 (71); Anal. calcd. for $C_{23}H_{19}N_6OSCl$ (462.96): C, 59.67; H, 4.14; N, 18.15. Found: C, 59.60; H, 4.10; N, 18.10 %.

**4-Phenylamino-5-phenyl-1,2,4-triazol-3-yl-N-phenyl-2-oxo-2-phenylethane thiohydrazonate (5d):** Yellow solid (1.22 g, 84 %); m.p. 245 ºC (dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3200, 1710, 1642; $^1H$ NMR (DMSO-$d_6$) δ: 6.57-8.38 (m, 20H), 10.95 (s, 2H, $D_2O$ exchangeable); MS m/z (%) 490 ($M^+$, 11), 413 (53), 384 (28), 267 (62), 235 (35), 158 (65), 105 (100), 76 (53), 51 (81); Anal. calcd. for $C_{28}H_{22}N_6OS$ (490.58): C, 68.55; H, 4.52; N, 17.13. Found: C, 67.95; H, 4.38; N, 17.10%.

**4-Phenylamino-5-phenyl-1,2,4-triazol-3-yl-N-phenyl-2-oxo-2-(2-naphthyl)ethane hydrazonothioate (5e):** Yellow solid (1.67 g, 62 %); m.p. 236 ºC (dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3134, 1676, 1635; $^1H$ NMR (DMSO-$d_6$) δ: 7.17-8.45 (m, 22H), 10.36 (s, 2NH, $D_2O$ exchangeable); MS m/z (%) 541 ($M^+$+1, 15), 540 ($M^+$, 22), 105 (100); Anal. calcd. for $C_{32}H_{24}N_6OS$: (540.65): C, 71.09; H, 4.47; N, 15.54. Found: C, 71.00; H, 4.45; N, 15.50 %.

**4-Phenylamino-5-phenyl-1,2,4-triazol-3-yl-N-phenyl-benzenecarbothiohydrazonate (5f):** Yellow solid (1.62 g, 70 %); m.p. 222 ºC (EtOH/DMF); IR (KBr, $\nu_{max}$, $cm^{-1}$): 3229, 3112, 1640; $^1H$ NMR (DMSO-$d_6$) δ: 7.23-8.03 (m, 20H), 11.26 (s, 2H, $D_2O$ exchangeable); MS m/z (%) 462 ($M^+$, 33), 447 (33), 378 (30), 323 (33), 301 (41), 296 (40), 278 (40), 210 (33), 202 (63), 169 (48), 131 (56), 103 (88), 90 (55), 76 (100), 52 (96); Anal. calcd. for $C_{27}H_{22}N_6S$ (462.58): C, 70.11; H, 4.79; N, 18.17. Found: C, 69.89; H, 4.71; N, 18.11 %.

**Synthesis of triazolo[4,3-b][1,2,4,5]tetrazine (7)**

**Method A:** To a mixture of 3-methylthio-4-phenylamino-5-phenyl-1,2,4-triazole (**2**) (0.71 g, 2.5 mmol) and the appropriate hydrazonoyl halide (**3**) (2.5 mmol) in absolute ethanol (20 mL), triethylamine (0.35 mL, 2.5 mmol) was added. The mixture was refluxed for 40-50 h till all methanethiol ceased to evolve, cooled and poured onto cold water. The solid product that precipitated was filtered, washed with water and finally crystallized from the appropriate solvent to give the respective 1,3,4,6-tetra substituted-1,4-dihydro[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazines (**7**).

When the above procedure was repeated using ethanolic sodium ethoxide solution, prepared from sodium metal (0.06 g, 2.5 mmol) and absolute ethanol (20 mL), in place of ethanolic triethylamine solution, the respective products were also obtained.

**Method B:** A solution of the appropriate thiohydrazonate (**5**) (2 mmol) in pyridine (10 mL) was refluxed for 20-30 h till hydrogen sulfide ceased to evolve then cooled and poured onto cold water. The solid that precipitated was filtered, washed with water and finally crystallized from the appropriate solvent to give the respective 1,3,4,6-tetra substituted-1,4-dihydro-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazines. By this method the products **7a-f** were prepared and proved identical in all respects with the respective products prepared by the above method A.

**3-Acetyl-1,4,6-triphenyl-[1,2,4]triazolo[4,3-b][1,2,4,5]-tetrazine (7a):** Yellow solid (1.26 g, 64 %), m.p. 246-248 ºC (EtOH/dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1705; $^1H$ NMR (DMSO-$d_6$) d: 2.55 (s, 3H), 7.27-8.48 (m, 15H); MS m/z (%) 394 ($M^+$, 8), 352 (23), 322 (32), 318 (14), 147 (56), 105 (100), 77 (81), 51 (65); Anal. calcd. for $C_{23}H_{18}N_6O$ (394.44): C 70.04; H, 4.60; N, 21.31. Found: C, 69.98; H, 4.61; N, 21.25 %.

**3-Acetyl-4,6-diphenyl-1-(tolyl)-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine (7b):** Yellow solid (1.33 g, 66 %), m.p. 235-238 ºC (EtOH/dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1695; $^1H$ NMR (DMSO-$d_6$) δ: 2.32 (s, 3H), 2.59 (s, 3H), 7.15 - 8.49 (m, 14H); MS m/z (%) 408 ($M^+$, 11), 393 (10), 365 (25), 324 (65), 317 (35), 143 (62), 91 (100), 77 (85), 51 (56); Anal. calcd. for $C_{24}H_{20}N_6O$ (408.47): C 70.57; H, 4.94; N, 20.57. Found: C, 70.50; H, 4.89; N, 20.52 %.

**3-Acetyl-4,6-diphenyl-1-(4-chlorophenyl)-[1,2,4]-triazolo[4,3-b][1,2,4,5]tetrazine (7c):** Red solid (1.50 g, 70 %), m.p. 265 ºC (EtOH/dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1700; $^1$H NMR (DMSO-$d_6$) δ: 2.55 (s, 3H), 7.18 - 8.41 (m, 14H); MS m/z (%) 430 ($M^+$ + 2, 8), 429 ($M^+$ + 1, 31), 428 ($M^+$, 23), 393 (43), 385 (56), 344 (25), 143 (66), 112, (65), 111, (11), 104 (100), 77 (73), 51 (46); Anal. calcd. for $C_{23}H_{17}N_6OCl$ (428.88): C 64.41; H, 4.00; N, 19.60. Found: C, 64.38; H, 3.89; N, 19.56 %.

**3-Benzoyl-1,4,6-triphenyl-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine (7d):** Buff solid (1.41 g, 55 %), m.p. 185 ºC (EtOH); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1705; $^1$H NMR (DMSO-$d_6$) δ: 6.95-8.38 (m, 20H); MS m/z (%) 457 ($M^+$+1, 5), 456 ($M^+$, 10), 455 (48), 352 (28), 322 (65), 146 (80), 103 (100), 77 (75), 51 (81); Anal. calcd. for $C_{28}H_{20}N_6O$ (456.51): C 73.67; H, 4.42; N, 18.41. Found: C, 72.92; H, 4.34; N, 18.25 %.

**3-(2-Naphthoyl)-1,4,6-triphenyl-[1,2,4]triazolo[4,3-b][1,2,4,5]tetrazine (7e):** Orange solid (1.52 g, 50 %), m.p. 218 ºC (EtOH); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1687; $^1$H NMR (DMSO-$d_6$) δ: 7.17-8.15 (m, 22H); MS m/z (%) 507 ($M^+$ + 1, 30), 506 ($M^+$, 22), 478 (25), 351 (36), 310 (63), 168 (35), 158 (62), 126 (33), 103 (65), 92 (12), 77 (100); Anal. calcd. for $C_{32}H_{22}N_6O$ (506.57): C 75.87; H, 4.38; N, 16.59. Found: C, 75.55; H, 4.35; N, 16.50 %.

**1,3,4,6-Tetraphenyl-[1,2,4]triazolo[4,3-b][1,2,4,5]-tetrazine (7f):** Buff solid (1.07 g, 50 %), m.p. 155-156 ºC (EtOH/dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1600; $^1$H NMR (DMSO-$d_6$) δ: 6.88-8.38 (m, 20H); MS m/z (%) 428 ($M^+$, 86), 311 (16), 296 (12), 269 (8), 219 (9), 126 (25), 117 (16), 103 (63), 77 (100), 51 (53); Anal. calcd. for $C_{27}H_{20}N_6$ (428.50): C 75.68; H, 4.70; N, 19.61. Found: C, 75.50; H, 4.66 N, 19.52 %.

**3-Acetyl-1-(4-nitrophenyl)-4,6-diphenyl[1,2,4]triazolo-[4,3-b][1,2,4,5]tetrazine (7g):** Pale brown solid (1.41 g, 64 %), m.p. 292-294 ºC (dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1701; $^1$H NMR (DMSO-$d_6$) δ: 2.33 (s, 3H), 7.17-8.40 (m, 14H); MS m/z (%) 440 ($M^+$+1, 14), 439 ($M^+$, 10), 411 (51), 397 (2), 369 (12), 335 (21), 293 (17), 240 (40), 219 (13), 136 (2), 119 (5), 103 (100), 77 (83), 51 (71); Anal. calcd. for $C_{23}H_{17}N_7O_3$ (439.44): C 62.87; H, 3.90; N, 22.31. Found: C, 62.85; H, 3.85; N, 22.25 %.

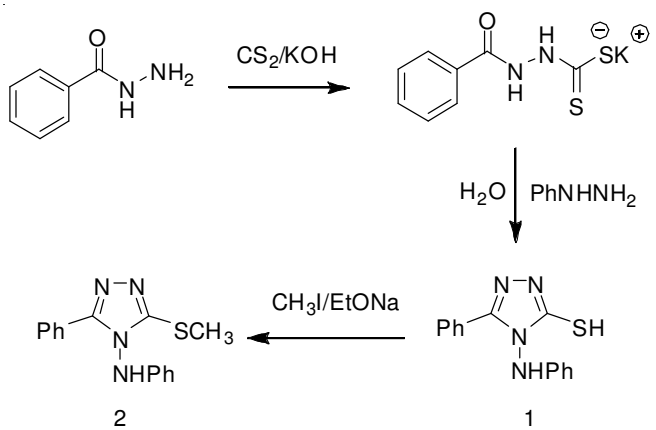
**3-Benzoyl-1-(4-nitrophenyl)-4,6-diphenyl[1,2,4]-triazolo[4,3-b][1,2,4,5]tetrazine (7h):** Buff solid (1.88 g, 75 %), m.p. 196 ºC (EtOH/DMF); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1698; $^1$H NMR (DMSO-$d_6$) δ: 7.25-8.51 (m, 19H); MS m/z (%) 502 ($M^+$+1, 27), 501 ($M^+$, 26), 424 (33), 397 (12), 369 (12), 144 (100), 123 (64) 91 (19), 77 (67), 51 (78); Anal. calcd. for $C_{28}H_{19}N_7O_3$ (501.51): C 67.06; H, 3.82; N, 19.55. Found: C, 67.0; H, 3.78; N, 19.45 %.

**1-(4-Nitrophenyl)-3-(2-naphthoyl)-4,6-diphenyl-[1,2,4]triazolo-[4,3-b][1,2,4,5]tetrazine (7i):** Pale brown solid (1.52 g, 55 %), m.p. 238 ºC (EtOH/dioxane); IR (KBr, $\nu_{max}$, $cm^{-1}$): 1695; $^1$H NMR (DMSO-$d_6$) δ: 7.24-8.49 (m, 21H); MS m/z (%) 551 ($M^+$, 11), 534 (36), 523 (20), 511 (25), 298 (28), 251 (20), 241 (23), 219 (20), 152 (15), 126 (23), 103 (66), 77 (100), 77 (83), 51 (28); Anal. calcd. for $C_{32}H_{21}N_7O_3$ (551.57): C 69.68; H, 3.84; N, 17.78. Found: C, 69.62; H, 3.81; N, 17.65%.

**Antimicrobial assay:** The antibacterial and antifungal activity assays were carried out at the Microbiology Division of Microanalytical Center of Cairo University using the diffusion plate method[15-18]. A bottomless cylinder containing a measured quantity (1 mL, mg/mL) of the sample is placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar broth) or fungal medium which has been heavily seeded with a spore suspension of the test organism. After incubation (24 h for bacteria and 5 days for fungi), the diameter of the clear zone of inhibition surrounding the sample is taken as measure of the inhibitory power of the sample against the particular test organism.

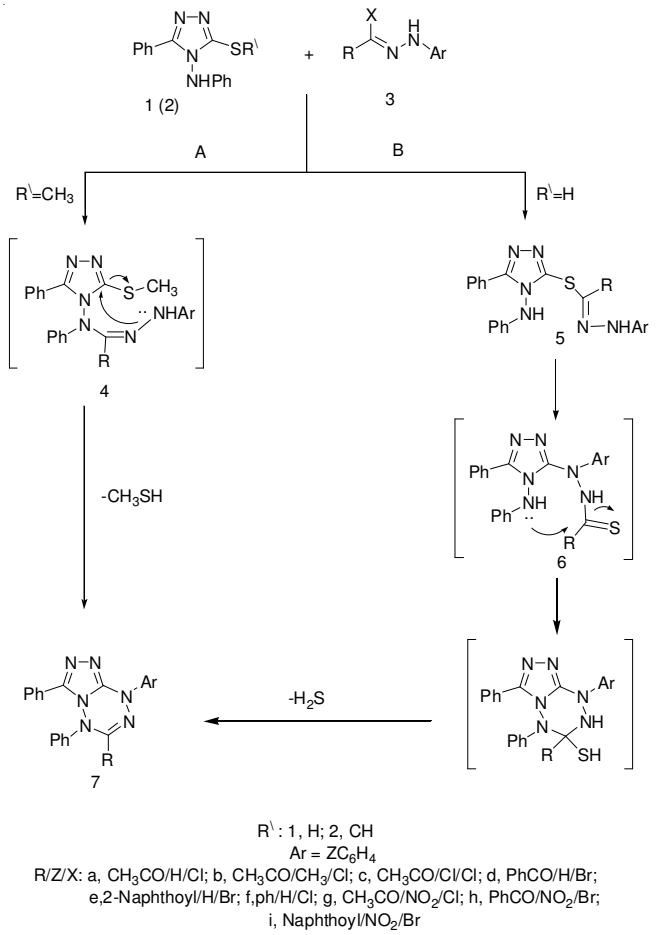
## RESULTS AND DISCUSSION

The required starting materials namely 4-pheylamino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**1**) and its 3-methylthio derivative (**2**). They were prepared in this work as depicted in **Scheme-I**. Thus, reaction of the benzoic acid hydrazide with carbon disulfide in alcoholic solution of potassium hydroxide gave crude potassium 3-benzoyldithiocarbazate, which upon refluxing with two equivalents of phenylhydrazine produced 4-phenylamino-5-phenyl-4*H*-[1,2,4]triazole-3-thiol (**1**). Methylation of the latter with methyl iodide in ethanol in the presence of sodium ethoxide afforded the 3-methylthio derivative (**2**) in 35 % yield. The structures of both **1** and **2** were evidenced by their spectra (MS, IR, $^1$H NMR) and microanalyses. For example, the IR spectrum of **1** reveals a broad band in the region 3300-3240 $cm^{-1}$ due to NH and SH group. Its $^1$H NMR shows in addition to the aromatic proton multiplet, two characteristic signals at δ 9.97 and 13.56 which are exchangeable with $D_2O$ assignable to NH and SH protons, respectively. The $^1$H NMR spectrum of **2** shows the presence of S-$CH_3$, NH protons as two singlets at δ 2.65 and 9.84, respectively. Its IR spectrum reveals an NH band in the region 3300-3200 $cm^{-1}$.




**Scheme-I:** Synthesis of 1,2,4-triazole-3-thiol and its 3-methylthio derivative

Refluxing equimolar quantities of each of the 4-phenyl-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol (**1**) and each of the hydrazonoyl halides (**3a-f**) in ethanol in the presence of triethyl-amine or sodium ethoxide yielded products that were identified as the respective thiohydrazonate esters (**5a-f**), respectively[12] (**Scheme-II**). The latter structures **5a-f** were evidenced by spectral and elemental analyses. For example, their $^1$H NMR spectra while they revealed the absence of the SH signal, they exhibited two NH singlet signals in the region δ 10.35-11.23.




**Scheme-II:** Synthesis of 1,2,4-triazolo[4,3-b][1,2,4,5]tetrazines

The formation of such thiohydrazonates (**5a-f**) is compatible with literature respects on reactions of hydrazonoyl halides with heterocyclic thiols[19]. Similar reactions of **1** with each **3g-i** which have a *p*-nitro group in the N-phenyl moiety under the same conditions, gave also in each case, one product as evidenced by tlc analysis. At first, it was anticipated that such reactions would yield the respective (4-pheylamino-5-phenyl-1,2,4-triazol-3-yl) thiohydrazonates or arylazo-5*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines derivatives by analogy to the reactions of 3 with 4-amino-5-phenyl-4*H*-[1,2,4]-triazole-3-thiol which were reported to give thiohydrazonates derivatives[20] or thiadiazines derivatives[14]. It is interesting to note that the products isolated from the reactions of **1** with **3g-i** were found to be free of sulfur. The structures of the isolated products were deduced from their elemental analyses, spectral (MS, IR and $^1$H NMR) data and their alternate synthesis. Their $^1$H NMR spectra in DMSO-$d_6$ display a common multiplet signal in the region δ 7.1-8.5 assignable to the aromatic protons. They revealed the absence of the N-NH and SH proton signals present in the spectrum of **1**. Their mass spectra showed peaks at m/z corresponding to [M$^+$], [M$^+$-R], [M$^+$-RC(NPh):NNHAr], [M$^+$-RC(:N)NH], [ArN] and [Ar] fragments.

Refluxing each of the thiohydrazonates (**5a-f**) in pyridine resulted in evolution of $H_2S$ and yielded, in each case, one product. The $^1$H NMR spectrum of this product showed the absence of both the singlet signals of NH protons present in the spectrum of **5** in the region δ 10.35-11.23, respectively. Their mass spectra showed, in addition to the expected molecular ion, peaks at m/z corresponding to [M$^+$ - R], [M$^+$ - RC(NPh):NNHAr], [M$^+$ -RC(:N)NH], [ArN] and [Ar] fragments. On the basis of these spectral data together with their microanalyses, the isolated products were assigned the 1,2,4-triazolo [4,3-b][1,2,4,5]-tetrazine structures (**7a-f**), respectively (**Scheme-II**).

To account for the formation of **7**, it is suggested that the thiohydrazonate **5** first underwent Smiles type rearrangement to the respective thiohydrazides **6**. The latter then underwent in situ cyclization *via* elimination of $H_2S$ to give **7** as end products (**Scheme-II**).

Reaction of **1** with each of the hydrazonoyl halides **3g-i** which have a *p*-nitrophenyl group in ethanol in the presence of triethylamine or sodium ethoxide yielded the respective **7g-i** directly as end products. In this case, it seems the *p*-nitro group facilitates the rearrangement of the thiohydrazonates and cyclization of the respective thiohydrazides.

Next, the reactions of 3-(methylthio)-N,5-diphenyl-4*H*-1,2,4-triazol-4-amine **2** with **3a-i** were investigated. Thus, refluxing a mixture of **2** and **3** in pyridine afforded, in each case, one product whose $^1$H NMR spectrum showed the absence of both the methylthio and aniline proton signals present at δ 2.65 and 9.84, respectively in the spectrum of the respective 3-(methylthio)-N,5-diphenyl-4*H*-1,2,4-triazol-4-amine (**2**). Such products proved identical in all respects (m.p., mixed m.p., IR) with those obtained above from **5**. As compound **2** cannot form thiohydrazonates with **3**, it is not unreasonable to conclude that reaction of **2** with **3** starts with the formation of the hydrazidines **4** which cyclized in situ to give **7** as end products (**Scheme-II**).

**Screening for antimicrobial activity:** Most of the synthesized compounds were tested in vitro against a Gram negative bacterium (*Escherichia coli* anaerobic), a Gram positive bacterium (*Staphylococcus albus*) and for antifungal activity against *Candida albicans* and *Aspergillus flavus*. The antibiotics ampicillin and tetracycline were used as references to evaluate the potency of the tested compounds under the same conditions. The solvent used was DMSO and the concentration of the sample used is 100 µg/mL. The results of antimicrobial

TABLE-1
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE SYNTHESIZED COMPOUNDS

| Compd. | Inhibition zone diameter (cm) | | | |
|---|---|---|---|---|
| | Gram (-) | Gram (+) | Fungi | |
| | *E. coli* | *S. albus* | *A. flavus* | *C. albicans* |
| **7a** | ++ | ++ | ++ | ++ |
| **7b** | ++ | ++ | ++ | ++ |
| **7c** | ++ | ++ | – | – |
| **7d** | ++ | ++ | – | – |
| **7e** | ++ | ++ | – | ++ |
| **7f** | ++ | ++ | – | ++ |
| **7g** | ++ | ++ | – | – |
| **7h** | ++ | ++ | ++ | ++ |
| **7i** | ++ | ++ | – | – |
| Tetracycline | +++ | +++ | | |
| Diflucan | | | ++ | ++ |

+ = Low activity, ++ = Moderate activity, +++ = High activity, – = No activity

activity are summarized in Table-1. The results revealed that all tested compounds exhibited moderate activity against the two tested bacteria species and *Candida albicans*.

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