Synthesis, Structure, and Biological Screening of Some Condensed Heterocyclic Compounds from 8-Chlorotetrazolo [5,1-f]-1,2,4-Triazine Precursor

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Abstract: 8-Chlorotetrazolo [5,1-f]-1,2,4-triazine (2) reacted with different compounds to yield a variety of novel condensed heterocyclic nitrogen compounds. The newly prepared structures were characterized by spectral data and screened for their antimicrobial activities against bacteria and fungi strains.

Keywords: 8-Chlorotetrazolo [5,1-f]-1,2,4-triazine, reactions, condensed heterocyclic compounds, antimicrobial assessment

1. INTRODUCTION

Many compounds consisting of the tetrazole nucleus have received much great attention because of their wide range of therapeutic and biological properties. They have emerged as antibacterial, antiproliferation, anticaner, and anticonvulsant agents. In this article, it is our intention to enlarge the area of the investigation towards tetrazolo-heterocycles using framework different reagents and expected interesting antimicrobial agents.

2. RESULTS AND DISCUSSION

The IR spectrum of tetrazol[5,1-f]-1,2,4-triazin-8(7H)-one\(^{5}\) showed a characteristic absorption bands at 3260 and 1670 cm\(^{-1}\) corresponding to the NH and CONH groups and the \(^{1}\)H NMR spectrum revealed exchangeable NH signal at 11.81 ppm. Compound 1 was treated with a mixture of phosphorus pentachloride and phosphorus oxychloride to yield the corresponding 8-chlorotetrazolo[5,1-f]-1,2,4-triazine (2) and was confirmed on the basis of its elemental analysis and devoid any bands for NH and CONH groups in IR region. The structure of compound 2 is a promising for the synthesis of diverse condensed heterocyclic nitrogen compounds (Schemes 1 and 2).

Treatment of cyclic imidoyl chloride 2 with the sodium salt of various amino acids namely: glycine, D-alanine, and β-alanine under reflux conditions produced the corresponding tetrazolotriazynilamino acids 3a,b and 4. The IR spectra of the latter compounds were confirmed on the basis absorption for OH, NH and CO groups. The \(^{1}\)H NMR spectrum of compound 3a showed a triplet signal at 4.56 ppm and exchangeable OH and NH protons as singlet signals at 11.21 and 8.12 ppm, respectively. The \(^{1}\)H NMR spectrum of compound 3b displayed a CH proton as a quartet signal at 4.42 ppm and exchangeable OH and NH protons as doublet signal at 1.51 ppm. The \(^{1}\)H NMR spectrum of compound 4 showed a triplet signal at 3.62 ppm assigned for CH\(_2\)CO and triplet signal at 3.22 ppm assigned for NCH\(_2\) beside exchangeable OH and NH protons. The mass spectra of 3a,b and 4 revealed the correct molecular ions which were supported by elemental analysis. The amino acid derivatives 3a,b and 4 were easily cyclized via 1,3 tautomerism in heating acetic anhydride in the presence of anhydrous sodium acetate to give imidazotetrazolotriazine derivatives 5a,b and tetrzolopyrimidinotriazine 6. The IR region of these compounds displayed the disappearance OH, NH and CO absorptions and showed of the absorption bands for amide groups at 1670-1680 cm\(^{-1}\). The \(^{1}\)H NMR spectra of compounds 5a,b and 6 showed the absence of OH and NH signals characteristic of the parent amino acids 3a,b and 4. These data together with the correct elemental analysis are compatible with 5a,b and 6 structures.

The reaction of chloro compound 2 with variety of aromatic acid hydrazides namely: benzoic, p-toluiic, p-chlorobenzoic, and p-nitrobenzoic afforded 3-aryl-1,2,4-triazo[4,3-d]tetrazolo [5,1-f]-1,2,4-triazine derivatives 7a-d, respectively. Elemental and spectra data of the latter compounds are consistent with the structure assigned to their compounds (cf. Experimental). More recently in the
literature, the aforementioned triazolotetrazotriazine structures were constructed by the reaction of 8-hydrazinotetrazole [5,1-f]-1,2,4-triazine with triethyl orthoformate or glacial acetic acid.

Scheme 1

Scheme 2
Cyclization of cyclic imidoyl chloride 2 using thiosemicarbazide in ethanol yielded 3-amino-1,2,4-triazolo derivative 8. The $^1$H NMR spectrum of compound 8 displayed two protons assigned exchangeable NH$_2$ as a singlet signal at 6.30 ppm. Additionally, the condensation of 8 with different aromatic aldehydes namely: benzaldehyde, $p$-tolualdehyde, and $p$-chlorobenzaldehyde leading to the arylidenes 9a-c, respectively. The IR spectra of the latter compounds possessed a characteristic absorption bands at 1620 and 1628 cm$^{-1}$ corresponding to the C=N groups. The $^1$H NMR spectra of compounds 9a-c revealed the presence azomethine (CH=N) at δ 8.52 and 9.02 ppm.

Furthermore, the reaction of chloro compound 2 with ammonium thiocyanate in ethanol produced the unisolable intermediate 10 that reacted in situ with phenyl isocyanate via 2+4 cycloaddition reaction to build 3-phenyl-2-thioxo-2,3-dihydropyrazolo[5',1':6,1]-1,2,4-triazino[4,5-\(d\)]-1,3,5-triazin-4-one(11). The IR spectrum of 11 showed the presence absorption bands at 1665 and 1270 cm$^{-1}$ attributed to CON and C=S, respectively.

The compound 2 and o-phenylenediamine dihydrochloride were successfully cyclized through the elimination of an ammonia molecule to the corresponding tetrazolo[5',1':6,1]-1,2,4-triazino[4,5-\(a\)]benzol[d]imidazole (12). Both IR and $^1$H NMR spectra showed no signals corresponding to the NH and NH$_2$ groups thus, confirming the structure of compound 12 whereas its mass spectrum showed the molecular ion peak at $m/z$ = 210.86 which was in agreement with molecular formula C$_9$H$_7$N$_7$ (m/z=211).

Also, interaction of compound 2 with anthranilic acid under fusion conditions, the expected quinazolinone derivative 14 was resulted as the only isolable product. The formation of 14 was explained by the formation of unisolable intermediate 13, undergoes intramolecular ring closure to form the exactly product 14, which IR and $^1$H NMR spectra exhibited devoid and OH and NH groups but showed CON absorption at 1670 cm$^{-1}$ in the IR region. The mass spectrum of 14 showed a peak corresponding to its molecular ion at $m/z$=239 (C$_{10}$H$_7$N$_7$O).

Moreover, treatment of two molar equivalents of cyclic imidoyl chloride 2 with one molar equivalent of each oxalic, malonic, and succinic acid dihydrazides caused a product in each case whose structure was verified from spectroscopic data. Its IR spectrum showed absorptions characteristic of 2NH and CON as well as two singlets attributed to 2NH each (exchangeable with D$_2$O) $^1$H NMR signals. Accordingly, the previously products were decisively assigned as bishydrazides 15a-c. The aforementioned products were in accordance with previous report with cyclic imidoyl chlorides.

On the other hand the structures 15a-c underwent cyclodehydration by phosphorus oxychloride to construct the bistriazolutetrazolotriazine derivatives 16a-c which showed IR absorptions characteristic of only a C≡N and, most importantly, lacked any NH and CON absorption bands characteristic of the parent compounds 15a-c. Furthermore, the mass spectra of 16a-c showed molecular ions in agreement with the assigned structures. Reaction of cyclic imidoyl chloride s with acid dihydrazides directly gave bistriazolo-structures.

### 3. ANTIMICROBIAL SCREENING

The newly prepared compounds were screened in vitro for their antimicrobial properties against Gram-positive (Staphylococcus aureus and Bacillus subtilis) and Gram-negative (Klebsiella pneumoniae and Escherichia coli) bacteria strains and (Aspergillums niger and Candida albican) fungi strains. Ampicillin and Clotrimazole were used as standard drugs for bacteria and fungi, respectively. The minimal inhibitory concentration (MIC, in μg. cm$^{-3}$), and the results are summarized in Table 1 showing that 4, 6, 7a, 9a and 12 exhibit an antimicrobial activity against S. aureus (25%); 3b, 6, 11, 12 and 16a against B. subtilis (25%); 7a, 8, 14, 15a and 16c against K. pneumoniae (50%); 5, 6, 11, 12 and 16a (50%) while compound 3b possessed activity against E. coli comparable to that ampicillin. Moreover, 3a, 7c, 8 and 14 possessed an antymycotic activity against A. niger (50%), and 4, 5, 11 and 12 against C. albican (50%) comparable to that clotrimazole. The compound of 15c showed lower activity than the reference standards (ampicillin and clotrimazole) against the test organisms.
Mamdouh A. M. Taha

Table 1. Antimicrobial activity of synthesized compounds (MIC/μg cm⁻²)

<table>
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<tr>
<th>Compound</th>
<th>Bacterial strain</th>
<th></th>
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<th>Fungal strain</th>
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4. Experimental

4.1. General

Melting points were measured with a Gallenkamp apparatus and are uncorrected. The reactions were followed up and the purification of products was carried out on pre-layer thickness 0.25mm; coated TLC plates Silica Gel-Merck), visualization the spots in Iodine. IR spectra were recorded (KBr) on a Shimadzu FT-IR 8101 PC infrared spectrophotometer. The ¹H NMR spectra were determined in DMSO-d₆ at 300 MHz on a Varian Mercury VX 300 NMR spectrometer and their chemical shifts (δppm) are reported using TMS as internal standard. Mass spectra were recorded on a HP model MS 5988 spectrometer at electron ionizing energy of 70 ev. Elemental analyses were performed by the microanalytical Unit, Cairo University, Egypt.

4.2. 8-Chlorotetrazolo[5,1-f]-1,2,4-triazine (2)

A suspension of tetrazolo[5,1-f]-1,2,4-triazin-8(7H)-one (1, 0.006 mol) and phosphorous pentachloride (0.006 mol) in phosphorous oxochloride (10 cm³) was heated under reflux on a water-bath for 2h. Then the mixture was cooled to room temperature and poured into crushed ice-water slowly. The obtained solid was filtered off, washed with cold water, dried and recrystallized from abs. ethanol to give 2, yield 0.84g (74.34%); m.p. 190-192 °C; ¹H NMR (DMSO-d₆) δ 6.25 ppm (s, 1H, CH₂); MS: m/z(%)= 156 (M⁺ Cl³5, 11)

Anal. Calcd. For C₅HClIN₆(156.5): C, 23.00; H, 0.64; N, 53.67. Found: C, 22.61; H, 1.04; N, 53.34%.

4.3. General procedure for the synthesis of tetrazolotrazinyl amino acids 3a,b and 4

The corresponding amino acids (0.006 mol) namely: glycine, D-alanine or β-alanine and sodium carbonate (0.06 mol) were dissolved in water (20 cm³). Then compound 2 (0.006 mol) was added to it and refluxed for 6h. The reaction mixture was left overnight at ambient temperature, and then treated with cold hydrochloric acid. The separated product was collected by filtration, dried and crystallized from abs. ethanol. The physico-chemical and spectra data of 3a,b and 4 the following:

4.4. 2-(Tetrazolo[5,1-f]-1,2,4-triazin-8-ylamino)acetic acid (3a)

Yield: 0.96g (77.05%); m.p. 270-272 °C; IR (KBr): ν=3300 (OH), 2445 (NH), 1720 cm⁻¹ (CO); ¹H NMR (DMSO-d₆): δ = 4.56 (s, 2H, CH₂), 6.26 (s, 1H, CH), 8.12 (s, 1H, NH, exchangeable with D₂O), 11.21 ppm (s, 1H, OH exchangeable with D₂O); MS: m/z (%), 196 (M⁺ Cl²9, 16).


4.5. 2-(Tetrazolo[5,1-f]-1,2,4-triazin-8-ylamino)propanoic acid (3b)

Yield: 0.93g (69.66%); m.p. 260-261 °C; IR (KBr): ν=3400 (OH), 2430 (NH), 1705 cm⁻¹ (CO); ¹H NMR (DMSO-d₆): δ = 1.51 (d, 3H, CH₃), 4.42 (q, 1H, CH), 6.20 (s, 1H, CH), 8.22 (s, 1H, NH,
exchangeable with D$_2$O), 10.83 ppm (s, 1H, OH exchangeable with D$_2$O); MS: m/z (%) = 211 (M$^+$+2, 20).

Anal. Calcd. For C$_8$H$_7$N$_7$O$_2$ (209): C, 34.45; H, 3.35; N, 46.89. Found: C, 34.49; H, 3.31; N, 47.01%.

4.6.3-(Tetrazolo[5,1-f]-1,2,4-triazin-8-ylamino)propanoic acid (4)

Yield: 0.88g (65.92%); m.p. 268-270 °C; IR (KBr): v=3300 (OH), 2395 (NH), 1720 cm$^{-1}$ (CO); $^1$H NMR (DMSO-$d_6$): $\delta$ = 3.22 (t, 2H, NCH$_2$), 3.62 (t, 2H, CH$_2$CO), 6.25 (s, 1H, CH), 8.24 (s, 1H, NH, exchangeable with D$_2$O), 11.02 ppm (s, 1H, OH exchangeable with D$_2$O); MS: m/z (%) = 209 (M$^+$ , 6).

Anal. Calcd. For C$_8$H$_7$N$_7$O$_2$ (209): C, 34.45; H, 3.35; N, 46.89. Found: C, 34.55; H, 3.48; N, 46.85%.

4.7. General procedure for the synthesis of imidazotetrazolotriazines 5a,b and tetrazolo pyrimidotriazine 6

A mixture of the appropriate (3a,b or 4, 0.005 mol) and acetic anhydride (20 cm$^3$), and anhydrous sodium acetate (0.42g, 0.005 mol) was heated under reflux for 4h. The solvent was removed under reduced pressure, the obtained residue was washed with water the recrystallized from abs. ethanol. The following data of the title compounds were prepared according to this procedure are described:

4.8. Imidazo[1,2-d]tetrazolo[5,1-f]-1,2,4-triazin-3(2H)-one (5a)

Yield: 0.58g (63.74%); m.p. 250-251 °C; IR (KBr): v=1670 cm$^{-1}$ (CON); $^1$H NMR (DMSO-$d_6$): $\delta$ = 6.66 (s, 2H, CH$_2$), 6.20 ppm (s, 1H, CH); MS: m/z (%) = 179 (M$^+$+2, 8).

Anal. Calcd. for C$_8$H$_7$N$_7$O$_2$ (177): C, 33.90; H, 1.70; N, 55.37. Found: C, 33.81; H, 1.74; N, 55.41%.

4.9. 2-Methylimidazo[1,2-d]tetrazolo[5,1-f]-1,2,4-triazin-3(2H)-one (5b)

Yield: 0.62g (68.13%); m.p. 240-242 °C; IR (KBr): v=1675 cm$^{-1}$ (CON); $^1$H NMR (DMSO-$d_6$): $\delta$ = 1.62 (d, 3H, CH$_3$), 4.60 (q, 1H, CH), 6.22 (s, 1H, CH) MS: m/z (%) = 191 (M$^+$ , 6).

Anal. Calcd. for C$_8$H$_7$N$_7$O$_2$ (191): C, 37.70; H, 2.62; N, 51.31. Found: C, 38.01; H, 2.44; N, 51.52%.

4.10. Tetrazolo[5,1-f]pyrimido[1,2-d]-1,2,4-triazin-4(2H, 3H)-one (6)

Yield: 0.52g (57.14%); m.p. 245-246 °C; IR (KBr): v=1680 cm$^{-1}$ (CON); $^1$H NMR (DMSO-$d_6$): $\delta$ = 3.10 (t, 2H, NCH$_2$), 4.05 (t, 2H, COCH$_2$), 6.28 ppm (s, 1H, CH) MS: m/z (%) = 191 (M$^+$ , 13).

Anal. Calcd. for C$_8$H$_7$N$_7$O$_2$ (191): C, 37.70; H, 2.62; N, 51.31. Found: C, 37.42; H, 3.01; N, 51.60%.

4.11. Reaction of 8-chlorotetrazolo[5,1-f]-1,2,4-triazine (2) with some acid hydrazides

4.11.1. General Procedure

A mixture of compound (2, 0.006 mol) and appropriate acid hydrazides (0.006 mol) namely: benzoic, p-toluic, p-chlorobenzoic, and p-nitrobenzoic in ethanol (30 cm$^3$) was refluxed for 3h. after cooling the mass product was filtered off and recrystallized from abs. ethanol to provide 7a-d.

4.12. 3-Phenyl-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (7a)

Yield: 0.91g (59.83%); m.p. 210-211 °C; IR (KBr): v=1620 cm$^{-1}$ (C=N); $^1$H NMR (DMSO-$d_6$): $\delta$ = 6.20 (s, 1H, CH), 7.02-8.52 ppm (m, 5H, ArH), MS: m/z (%) = 240 (M$^+$+2, 21).

Anal. Calcd. for C$_{10}$H$_9$N$_8$ (238): C, 50.42; H, 2.52; N, 47.06. Found: C, 50.55; H, 2.31; N, 46.82%.

4.13. 3-p-Tolyl-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (7b)

Yield: 0.84g (52.17%); m.p. 225-226 °C; IR (KBr): v=1625 cm$^{-1}$ (C=N); $^1$H NMR (DMSO-$d_6$): $\delta$ = 2.30 (s, 3H, CH$_3$), 6.25(s, 1H, CH), 7.10-8.61 ppm (m, 4H, ArH), MS: m/z (%) = 252 (M$^+$ , 17).

Anal. Calcd. for C$_{11}$H$_9$N$_8$ (252): C, 52.38; H, 3.18; N, 44.44. Found: C, 52.11; H, 3.22; N, 44.49%.

4.14. 3-p-Chlorophenyl-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (7c)

Yield: 0.94g (54.02%); m.p. 240-241 °C; IR (KBr): v=1630 cm$^{-1}$ (C=N); $^1$H NMR (DMSO-$d_6$): $\delta$ = 6.20 (s, 1H, CH), 7.17-8.51 ppm (m, 4H, ArH), MS: m/z (%) = 237 (M$^+$ Cl$^{35}$ , 8).

International Journal of Advanced Research in Chemical Science (IJARCS)
Mamdouh A. M. Taha

Anal. Calcd. for C_{10}H_{3}ClN_{8} (272.5): C, 44.04; H, 1.85; N, 41.10. Found: C, 44.10; H, 1.81; N, 44.51%.

4.15. 3-p-Nitrophenyl-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (7d)

Yield: 1.11g (61.33%); m.p. 270-272 °C; IR (KBr): \(\nu=1605 \text{ cm}^{-1} (\text{C=\text{N}})\); \(^1\text{H NMR} (\text{DMSO-d}_6): \delta = 6.27 \text{ (s, 1H, CH)}, 7.20-8.15 \text{ ppm (m, 4H, ArH)}, \text{MS: m/z (%)} = 284 \text{ (M}^+1, 10). \)

Anal. Calcd. for C_{10}H_{3}N_{2}O_{2} (283): C, 42.40; H, 1.77; N, 44.52. Found: C, 42.61; H, 1.61; N, 44.20%.

4.16. 3-Amino-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (8)

A mixture of compound 2 (0.006 mol) and thiosemicarbazide (0.006 mol) in abs. ethanol was refluxed for 4h. The resulting product was cooled to ambient temperature and filtered, and recrystallized from abs. ethanol to yield 8 in 0.81g (71.68%); m.p. 235\text{o}C; IR (KBr): \(\nu=3420, 3390 \text{ cm}^{-1} (\text{NH}_{2}); \) \(^1\text{H NMR} (\text{DMSO-d}_6): \delta = 6.20 \text{ (s, 1H, CH)}, 6.30 \text{ ppm (s, 2H, NH}_{2}; \text{exchangeable with D}_2\text{O), MS: m/z} (\%) = 177 \text{ (M}^+, 22). \)

Anal. Calcd. for C_{10}H_{3}N_{2} (177): C, 27.12; H, 1.70; N, 71.19. Found: C, 26.89; H, 1.95; N, 70.82%.

4.17. Preparation of 3-(N-arylideneamino)-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazines (9a-c)

Corresponding aromatic aldehyde (0.006 mol) namely: benzaldehyde, p-toluualdehyde, and p-chlorobenzaldehyde was added to a solution of 8 (0.006 mol) in abs. ethanol (20 cm^3). The reaction mixture was refluxed for 2h. The formed solid was collected by filtration, and recrystallized from abs. ethanol to result the title compounds 9a-c. The physico-chemical and spectra data as follows:

4.18. 3-(N-Benzylideneamino)-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (9a).

Yield: 0.92g (61.33%); m.p. 190-192 °C; IR (KBr): \(\nu=1620 \text{ cm}^{-1} (\text{C=\text{N}}); \) \(^1\text{H NMR} (\text{DMSO-d}_6): \delta = 6.22 \text{ (s, 1H, CH)}, 7.00-8.15 \text{ ppm (m, 5H, ArH)}, 8.52 \text{ ppm (s, 1H, CH=CH=N), MS: m/z} (\%) = 265 \text{ (M}^+, 30). \)

Anal. Calcd. for C_{11}H_{12}N_{2}O_{2} (265): C, 49.81; H, 2.64; N, 47.55. Found: C, 50.11; H, 2.52; N, 46.40%.

4.19. 3-(N-P-Tolylideneamino)-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (9b).

Yield: 1.21g (76.58%); m.p. 185-187 °C; \(^1\text{H NMR} (\text{DMSO-d}_6): \delta = 2.33 \text{ (s, 3H, CH}_{3}; \) 6.25 \text{ (s, 1H, CH)}, 7.20-8.15 \text{ ppm (m, 4H, ArH)}, 8.52 \text{ ppm (s, 1H, CH=N), MS: m/z} (\%) = 281 \text{ (M}^+2, 9). \)

Anal. Calcd. for C_{12}H_{10}N_{2}O_{2} (279): C, 51.61; H, 3.23; N, 45.16. Found: C, 51.42; H, 3.72; N, 45.34%.

4.20. 3-(N-P-Chlorobenzylideneamino)-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine (9c).

Yield: 1.11g (65.68%); m.p. 197-199 °C; IR (KBr): \(\nu=1628 \text{ cm}^{-1} (\text{C=\text{N}}); \) \(^1\text{H NMR} (\text{DMSO-d}_6): \delta = 6.21 \text{ (s, 1H, CH)}, 7.27-8.20 \text{ ppm (m, 4H, ArH)}, 9.02 \text{ ppm (s, 1H, CH=CH=N).} \)

Anal. Calcd. for C_{11}H_{8}ClN_{2}O_{2} (299.5): C, 44.07; H, 2.00; N, 42.07. Found: C, 43.88; H, 2.34; N, 42.13%.

4.21. 3-Phenyl-2-thioxo-2,3-dihydrotetrazolo[5',1*:6,1]-1,2,4-triazino[4,5-a]-1,3,5-triazin-4-one (11)

The solution of ammonium thiocyanate (0.006 mol) in abs. ethanol was added to a stirred solution of 2 (0.006 mol) in abs. ethanol. The reaction mixture was stirred for 30 min at ambient temperature. Ammonium chloride was precipitated during the reaction, filtration and phenyl isocyanate (0.006 mol) was added to the filtrate ad the reaction mixture was heated under reflux for 30 min after cooling the precipitated was collected and crystallized from abs. ethanol, and dried, yield: 1.32g (69.47%); m.p. 235-237 °C; IR (KBr): \(\nu=1665 \text{ (CON)}, 1270 \text{ cm}^{-1} (\text{C=S}); \) \(^1\text{H NMR} (\text{DMSO-d}_6): \delta = 6.25 \text{ (s, 1H, CH)}, 7.30-8.35 \text{ ppm (m, 5H, ArH)}, \text{MS: m/z} (\%) = 300 \text{ (M}^+2, 19). \)

Anal. Calcd. for C_{11}H_{8}N_{2}OS (298): C, 44.30; H, 2.01; N, 37.58. Found: C, 44.72; H, 1.99; N, 37.99%.

4.22. Tetrazolo[5',1*:6,1]-1,2,4-triazino[4,5-a]benzo[d] imidazole (12)

A mixture of compound 2 (0.006 mol) and o-phenylenediamine dihydrochloride (0.006 mol) was fused in an oil bath at 190-191 °C for an hour and then allowed to attain ambient temperature and added to 20 cm^2 of cold water. The mass product obtained was filtered off, dried, and recrystallized...
4.23. Tetrazolo[5',1'-6,1]-1,2,4-triazin-4,5-b]quinazolin-8-one (14)

A mixture of 2 (0.006 mol) and anthranilic acid (0.006 mol) was heated in oil bath at 190-191 °C for an hour and then allowed to cool down at room temperature; the obtained residue was crystallized from abs. ethanol to result 14. Yield: 0.94g (61.44%); m.p. 180-182 °C; IR (KBr): ν=1670 (CON), 1H NMR (DMSO-d6): δ = 6.15, 6.20 (2s, 1H each, 2CH); 11.90, 12.20 ppm (2s, 2H each, 2 NH each, exchangeable with D2O); MS: m/z (%) = 239 (M⁺ , 22).

Anal. Calcd. for C10H6N4O2 (239): C, 50.21; H, 2.09; N, 41.00. Found: C, 49.91; H, 2.11; N, 41.33%.


To a solution of 2 (0.006 mol) in 15 cm³ abs. ethanol, a solution of (0.006 mol) corresponding acid dihydrazide namely: oxalic, malonic and succinic in 15 cm³ abs. ethanol was gradually added, and the mixture was heated under reflux at 100 °C for an hour. The product which separated upon cooling was filtered off, and crystallized from mixture of water and abs. ethanol to afford the products 15a-c.

4.25. Oxalyl bis{(tetrazolo[5,1-f]-1,2,4-triazin-8-yl) hydrazide} (15a)

Yield: 1.67g (72.93%); m.p. 230-232 °C; IR (KBr): ν=3285, 3280 (2NH), 1665 cm⁻¹ (CON), 1H NMR (DMSO-d6): δ = 6.15, 6.20, (2s, 1H each, 2CH); 11.90, 12.20 ppm (2s, 2H each, 2 NH each, exchangeable with D2O); MS: m/z (%) = 360 (M⁺+2, 27).

Anal. Calcd. for C16H12N6O2 (358): C, 57.82; H, 3.92; N, 38.26. Found: C, 57.73; H, 4.01; N, 38.55%.

4.26. Malonyl bis{(tetrazolo[5,1-f]-1,2,4-triazin-8-yl) hydrazide} (15b)

Yield: 1.45g (60.92%); m.p. 220-222 °C; 1H NMR (DMSO-d6): δ = 4.41 (s, 2H, CH2); 6.20 (s, 2H, 2CH), 11.85, 12.30 ppm (2s, 2H each, 2NH each, exchangeable with D2O); MS: m/z (%) = 372 (M⁺ , 10).


4.27. Succinyl bis{(tetrazolo[5,1-f]-1,2,4-triazin-8-yl) hydrazide} (15c)

Yield: 1.34g (54.25%); m.p. 240-243 °C; IR (KBr): ν=3275, 3290 (2NH), 1690 cm⁻¹ (CON), 1H NMR (DMSO-d6): δ = 3.25, 3.40 (2t, 2H each, CH2 each); 6.25 (s, 2H, 2CH), 12.01, 12.20 ppm (2s, 2H each, 2NH each, exchangeable with D2O); MS: m/z (%) = 387 (M⁺+1, 9).

Anal. Calcd. for C16H12N6O2 (386): C, 31.09; H, 2.59; N, 57.82. Found: C, 30.82; H, 2.61; N, 57.82%.

4.28. Synthesis of 16a-c: General Procedure

The respective of 15a-c (0.006 mol) was treated with 20 cm³ phosphorus oxychloride and heated under reflux for 2h. After attaining ambient temperature, the mixture was poured onto a cold saturated solution of sodium bicarbonate and the crude solid which separated was filtered off, washed with water, dried, and crystallized from abs. ethanol to yield the products 16a-c.

4.29. Bis {1,2,4-triazolo[4,3-d]tetrazolo [5,1-f]-1,2,4-triazin-3-yl} (16a)

Yield: 1.62g (78.64%); m.p. 240-242 °C; IR (KBr): ν=1610 cm⁻¹ (C≡N), MS: m/z (%) = 323 (M⁺+1, 10).

Anal. Calcd. for C33H14N16 (322): C, 29.81; H, 0.62; N, 69.57. Found: C, 29.90; H, 1.05; N, 70.01%.

4.30. Bis {1,2,4-triazolo[4,3-d]tetrazolo [5,1-f]-1,2,4-triazin-3-yl} methane (16b)

Yield: 1.72g (80.00%); m.p. 230-232 °C; 1H NMR (DMSO-d6): δ = 4.46, (s, 2H, CH2); 6.25 ppm (s, 2H each, 2CH); MS: m/z (%) = 336 (M⁺ , 27).

4.31. Bis {1,2,4-triazolo[4,3-d]tetrazolo [5,1-f]-1,2,4-triazin-3-yl} ethane (16c)
 Yield: 1.76g (78.57%); m.p. 250-271 °C; IR (KBr): ν=1620 cm⁻¹ (C=N), MS: m/z (%) = 351 (M⁺+1, 19).
 Anal. Calcd. for C₁₅H₈N₁₆ (350): C, 34.29; H, 1.71; N, 64.00. Found: C, 34.09; H, 1.95; N, 64.24%.

5. ANTIMICROBIAL SCREENING
 Solutions of the test compounds, ampicillin trihydrate and clotrimazole were prepared in DMSO at a concentration of 100 µg/cm³. Twofold dilution of the compounds were prepared (800, 400, …, 6.25 g/cm³). The microorganism suspensions at 10⁶ colony Formin Unit 1 cm⁻³ (CFU/cm³) concentration were inoculate to the corresponding wells. Plates were incubated at 36°C for 24 to 28h in the incubation chamber. The minimal inhibitory concentration (MIC) were determined. Controls with DMSO and infected media were also investigated.

6. CONCLUSION
 The foregoing results demonstrated the utility of 8-chlorotetrazolo [5,1-f]-1,2,4-triazine as synthons for the construction of some condensed heterocyclic nitrogen structures by different cyclization reagents. The antibacterial and antifungi activities of the synthesized compounds were even comparable to ampicillin and clotrimazole.

REFERENCES
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