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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[1-10] much great attention because of their wide range of therapeutic and biological properties.[11,12] They have emerged as antibacterial,[2-8,13] antiproliferation,[14] anticancer,[14] and anticonvulsant[15] agents. In this article, it is our intention to enlarge the area of the investigation towards tetrazolo-heterocycles using framework different reagants and expected interesting antimicrobial agents.

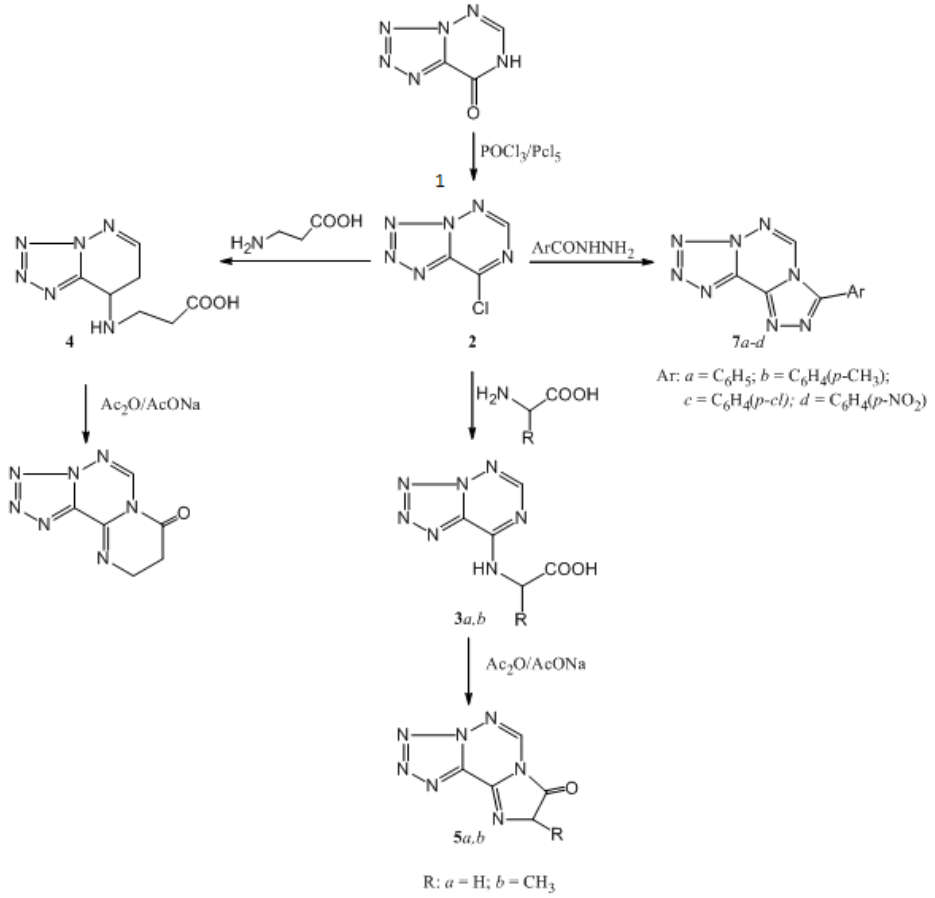
2. RESULTS AND DISCUSSION

The IR spectrum of tetrazolo[5,1-*f*]-1,2,4-triazin-8(7H)-one[4] **(1)** showed a characteristic absorption bands at 3260 and 1670 cm^{-1} corresponding to the NH and CONH groups and the ^1H NMR spectrum revealed exchangeable NH signal at 11.81 ppm. Compound **1** was treated with a mixture of phosphorous pentachloride and phosphorous 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**).

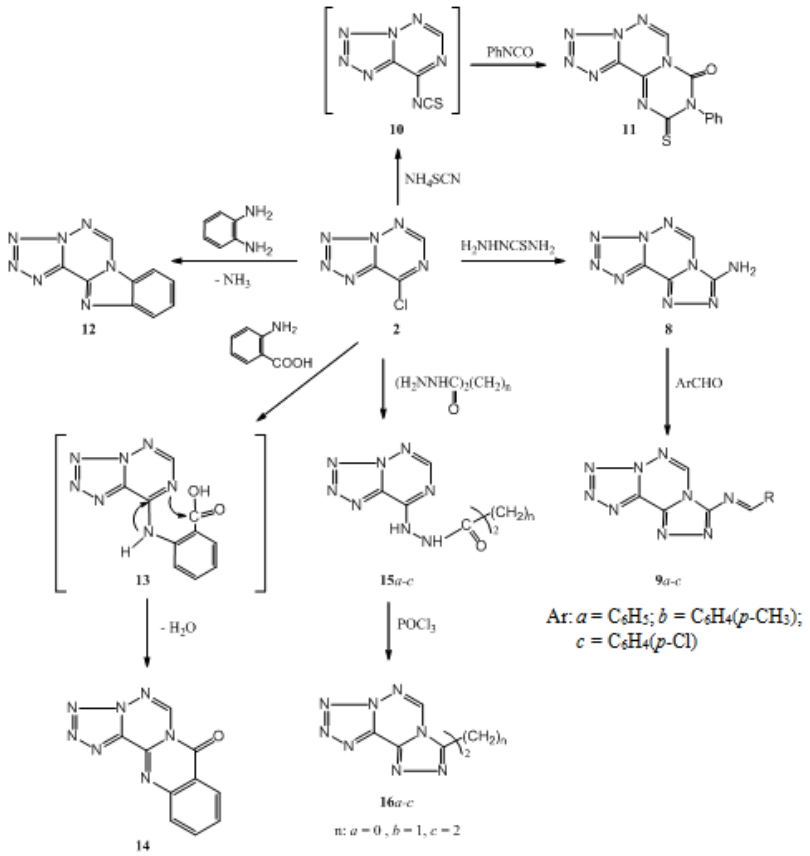
Treatment of cyclic imidoyl chloride **2** with the sodium salt of various amino acids namely: glycine, D-alanine, and β-alanine under reflux conditions produced the corresponding tetrazolotriazinylamino acids **3***a,b* and **4**. The IR spectra of the latter compounds were confirmed on the basis absorption for OH, NH and CO groups. The ^1H NMR spectrum of compound **3***a* displayed CH_2 protons as a singlet signal at 4.56 ppm and exchangeable OH and NH protons as singlet signals at 11.21 and 8.12 ppm, respectively. The ^1H NMR spectrum of compound **3***b* displayed a CH proton as a quartet signal at 4.42 ppm and CH_3 protons as a doublet signal at 1.51 ppm. The ^1H NMR spectrum of compound **4** showed a triplet signal at 3.62 ppm assigned for CH_2CO and triplet signal at 3.22 ppm assigned for NCH_2 beside exchangeable OH and NH protons. The mass spectra of **3***a,b* and **4** revealed the correct molecular ions which were supported by elemental analysis. The amino acid derivatives **3***a,b* and **4** were easily cyclized[16] *via* 1,3 tautomerism in heating acetic anhydride in the presence of anhydrous sodium acetate to give imidazotetrazolotriazine derivatives **5***a,b* and tetrazolopyrimidinotriazine **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 ^1H NMR spectra of compounds **5***a,b* and **6** showed the absence of OH and NH signals characteristic of the parent amino acids **3***a,b* and **4**. These data together with the correct elemental analysis are compatible with **5***a,b* and **6** structures.

The reaction of chloro compound **2** with variety of aromatic acid hydrazides namely: benzoic, *p*-toluic, *p*-chlorobenzoic, and *p*-nitrobenzoic afforded 3-aryl-1,2,4-triazolo[4,3-*d*]tetrazolo [5,1-*f*]-1,2,4-triazine derivatives **7***a-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,[4] 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.



Scheme1



Scheme2

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 **9***a-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 **9***a-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-dihydrotetrazolo[5`,1`:6,1]-1,2,4-triazino[4,5-*a*]-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*]benzo[*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 moleculer ion peak at *m/z* = 210.86 which was in agreement with molecular formula $C_9H_5N_7$ (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 ($C_{10}H_5N_7O$).

Moreover, treatment of two molar equivalents of cyclic imidoyl chloride **2** with one molor 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_2O) ^{1}H NMR signals. Accordingly, the previously products were decisively assigned as bishydrazides **15***a-c*. The aforementioned products were in accordance with previous report[5] with cyclic imidoyl chlorides.

On the other hand the structures **15***a-c* underwent cyclodehydration by phosphorus oxychloride to construct the bistriozolotetrazolotriazine derivatives **16***a-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 **15***a-c*. Furthermore, the mass spectra of **16***a-c* showed molecular ions in agreement with the assigned structures. Reaction[17] 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 antimircrobial properties against Gram-positive (*Staphylococcus aureus and Bacillus substilis*) and Gram-negative (*Klebsiella poneumoniae 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[18] (*MIC*, in μg. cm^{-3}), and the results are summarized in **Table 1** showing that **4, 6, 7*a*, 9*a*** and **12** exhibit an antimicrobial activity against *S. aureus* (25%); **3*b*, 6, 11, 12** and **16*a*** against *B. subtilis* (25%); **7*a*, 8, 14, 15*a*** and **16*c*** against *K. poneumoniae* (50%); **5, 6, 11, 12** and **16*a*** (50%) while compound **3b** possessed activity against *E. coli* comparable to that ampicillin. Moreover, **3*a*, 7*c*, 8** and **14** possessed an antimycotic activity against *A. niger* (50%), and **4, 5, 11** and **12** against *C. albican* (50%) comparable to that clotrimazole. The compound of **15*c*** showed lower activity than the reference standards (ampicillin and clotrimazole) against the test organisms.

Table1. *Antimicrobial activity of synthesized compounds (MIC/μg cm^{-3})*

Compound	*Bacterial strain*				*Fungal strain*	
	S.aureus	*B.subtitis*	*K.pneumoniae*	*E.coli*	*A.niger*	*C.albican*
3a	100	>200	100	>200	25	100
3b	100	50	100	25	100	100
4	50	100	100	100	100	25
5	>200	100	100	50	100	25
6	50	50	100	50	100	100
7a	50	100	50	>200	100	100
7c	100	>200	>200	100	25	100
8	100	100	50	100	25	100
9a	50	100	100	100	100	100
11	100	50	100	50	100	25
12	50	50	100	50	100	25
14	100	100	50	100	25	100
15a	100	100	50	100	100	100
15c	100	100	100	100	100	100
16a	100	50	100	50	100	100
16c	100	100	50	>200	100	100
Ampicillin	12.5	12.5	25	25	--	-
Clotrimazole	--	--	--	--	12.5	12.5

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), visualizin the spots in Iodine. IR spectra were recorded (KBr) on a Shimadzu FT-IR 8101 PC infrared spectrophotometer. The ^{1}H NMR spectra were determined in DMSO(d_6) at 300 MHz on a Varian Mercury VX 300 NMR spectrometer and their chemical shifts (δ/ppm) 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. Elemtenal 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[4] (**1**, 0.006 mol) and phosphorous pentachloride (0.006 mol) in phosphorous oxochloride (10 cm^3) was heated under reflux on a water-bath for 2h. Then the mixture was cooled to room temperatue 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 ^{o}C; ^{1}H NMR (DMSO-d_6) δ: 6.25 ppm (s, 1H, CH); MS: *m.z*(%)= 156 (M^+ Cl^{35}, 11)

Anal. Calcd. For C_3HClN_6(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 cm^3). 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 filteration, dried and crystallized from abs. ethanol. The physico-chemical and spectra data of **3***a,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 ^{o}C; IR (KBr): ν=3300 (OH), 2445 (NH), 1720 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6): δ = 4.56 (s, 2H, CH_2), 6.26 (s, 1H, CH), 8.12 (s, 1H, NH, exchangeable with D_2O), 11.21 ppm (s, 1H, OH exchangeable with D_2O); MS: *m/z* (%), 196 (M^+ + 1, 16).

Anal. Calcd. for $C_5H_5N_7O_2$ (195): C, 30.77; H, 2.56; N, 50.26. Found: C, 31.11; H, 2.41; N, 50.11%.

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 ^{o}C; IR (KBr): ν=3400 (OH), 2430 (NH), 1705 cm^{-1} (CO); ^{1}H NMR (DMSO-d_6): δ = 1.51 (d, 3H, CH_3), 4.42 (q, 1H, CH), 6.20 (s, 1H, CH), 8.22 (s, 1H, NH,

exchangeable with D_2O), 10.83 ppm (s, 1H, OH exchangeable with D_2O); MS: *m/z* (%) = 211 (M^++2, 20).

Anal. Calcd. For $C_6H_7N_7O_2$ (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 ^oC; IR (KBr): ν=3300 (OH), 2395 (NH), 1720 cm^{-1} (CO); ^1H NMR (DMSO-d_6): δ = 3.22 (t, 2H, NCH_2), 3.62 (t, 2H, CH_2CO), 6.25 (s, 1H, CH), 8.24 (s, 1H, NH, exchangeable with D_2O), 11.02 ppm (s, 1H, OH exchangeable with D_2O); MS: *m/z* (%) = 209 (M^+, 6).

Anal. Calcd. For $C_6H_7N_7O_2$ (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 (**3***a*,*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 ^oC; IR (KBr): ν=1670 cm^{-1} (CON); ^1H NMR (DMSO-d_6): δ = 4.66 (s, 2H, CH_2), 6.20 ppm (s, 1H, CH), MS: *m/z* (%) = 179 (M^++2, 8).

Anal. Calcd. for $C_5H_3N_7O$ (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 ^oC; IR (KBr): ν=1675 cm^{-1} (CON); ^1H NMR (DMSO-d_6): δ = 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 $C_6H_5N_7O$ (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 ^oC; IR (KBr): ν=1680 cm^{-1} (CON); ^1H NMR (DMSO-d_6): δ = 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 $C_6H_5N_7O$ (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 **7***a-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 ^oC; IR (KBr): ν=1620 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ = 6.20 (s, 1H, CH), 7.02-8.52 ppm (m, 5H, ArH), MS: *m/z* (%) = 240 (M^++2, 21).

Anal. Calcd. for $C_{10}H_6N_8$ (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 (7*b*)

Yield: 0.84g (52.17%); m.p. 225-226 ^oC; IR (KBr): ν=1625 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ = 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 $C_{11}H_8N_8$ (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 (7*c*)

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

Anal. Calcd. for $C_{10}H_5ClN_8$ (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 (7*d*)

Yield: 1.11g (61.33%); m.p. 270-272 ^oC; IR (KBr): ν=1605 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ = 6.27 (s, 1H, CH), 7.20-8.15 ppm (m, 4H, ArH), MS: *m/z* (%) = 284 (M^++1, 10).

Anal. Calcd. for $C_{10}H_5N_9O_2$ (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 (25 cm^3) 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. 220-221 ^oC; IR (KBr): ν=3420, 3390 cm^{-1} (NH_2); ^1H NMR (DMSO-d_6): δ = 6.20

(s, 1H, CH), 6.30 ppm (s, 2H, NH_2, exchangeable with D_2O), MS: *m/z* (%) = 177 (M^+, 22).

Anal. Calcd. for $C_4H_3N_9$ (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-tolualdehyde, 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 **9***a-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 (9*a*).

Yield: 0.92g (61.33%); m.p. 190-192 ^oC; IR (KBr): ν=1620 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ = 6.22 (s, 1H, CH), 7.00-8.15 ppm (m, 5H, ArH),8.52 ppm (s, 1H, CH=N) MS: *m/z* (%) = 265 (M^+, 30).

Anal. Calcd. for $C_{11}H_7N_9$ (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 (9*b*)

Yield: 1.21g (76.58%); m.p. 185-187 ^oC; ^1H NMR (DMSO-d_6): δ = 2.33 (s, 3H, CH_3), 6.25 (s, 1H, CH), 7.20-8.15 (m. 4H, ArH), 8.52 ppm (s, 1H, CH=N), MS: *m/z* (%) = 281 (M^++2, 9).

Anal. Calcd. for $C_{12}H_9N_9$ (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 (9*c*)

Yield: 1.11g (65.68%); m.p. 197-199 ^oC; IR (KBr): ν=1628 cm^{-1} (C=N); ^1H NMR (DMSO-d_6): δ = 6.21 (s, 1H, CH), 7.27-8.20 (m. 4H, ArH), 9.02 ppm (s, 1H, CH=N).

Anal. Calcd. for $C_{11}H_6ClN_9$ (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, filteration 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 ^oC; IR (KBr): ν=1665 (CON), 1270 cm^{-1} (C=S); ^1H NMR (DMSO-d_6): δ = 6.25 (s, 1H, CH), 7.30-8.35 ppm (m, 5H, ArH); MS: *m/z* (%) = 300 (M^++2, 19).

Anal. Calcd. for $C_{11}H_6N_8OS$ (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 ^oC for an hour and then allowed to attain ambient temperature and added to 20 cm^3 of cold water. The mass product obtained was filtered off, dried, and recrystallized

from abs. ethanol to produce **12**. yield: 0.82g (65.08%); m.p. 210-212 ^{o}C; ^{1}H NMR (DMSO-d_6): δ = 6.24 (s, 1H, CH), 7.20-8.12 ppm (m, 4H, ArH); MS: *m/z* (%) = 210.86 (M^+ , 26).

Anal. Calcd. for $C_9H_5N_7$ (211): C, 51.18; H, 2.37; N, 46.45. Found: C, 50.98; H, 2.11; N, 46.31%.

4.23. Tetrazolo[5`,1`:6,1]-1,2,4-triazino[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 ^{o}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. 220-222 ^{o}C; IR (KBr): ν=1670 (CON), ^{1}H NMR (DMSO-d_6): δ = 6.27 (s, 1H, CH), 7.05-8.10 ppm (m, 4H, ArH); MS: *m/z* (%) = 239 (M^+ , 22).

Anal. Calcd. for $C_{10}H_5N_7O$ (239): C, 50.21; H, 2.09; N, 41.00. Found: C, 49.91; H, 2.11; N, 41.33%.

4.24. Synthesis of 15*a-c*: *General Procedure*

To a solution of **2** (0.006 mol) in 15 cm^3 abs. ethanol, a solution of (0.006 mol) corresponding acid dihydrazide namely: oxalic, malonic and succinic in 15 cm^3 abs, ethanol was gradually added, and the mixture was heated under reflux at 100 ^{o}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 **15***a-c*.

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

Yield: 1.67g (72.93%); m.p. 230-232 ^{o}C; IR (KBr): ν=3285, 3280 (2NH), 1665 cm^{-1} (CON), ^{1}H NMR (DMSO-d_6): δ = 6.15, 6.20, (2s, 1H each, 2CH); 11.90, 12.20 ppm (2s, 2H each, 2 NH each, exchangeable with D_2O); MS: *m/z* (%) = 360 (M^++2, 27).

Anal. Calcd. for $C_8H_6N_{16}O_2$ (358): C, 26.82; H, 1.68; N, 62.57. Found: C, 27.25; H, 1.71; N, 62.67%.

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

Yield: 1.45g (60.92%); m.p. 220-222 ^{o}C; ^{1}H NMR (DMSO-d_6): δ = 4.41 (s, 2H, CH_2); 6.20 (s, 2H, 2CH), 11.85, 12.30 ppm (2s, 2H each, 2NH each, exchangeable with D_2O); MS: *m/z* (%) = 372 (M^+ , 10).

Anal. Calcd. for $C_9H_8N_{16}O_2$ (372): C, 29.03; H, 2.15; N, 60.22. Found: C, 28.86; H, 2.22; N, 60.52%.

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

Yield: 1.34g (54.25%); m.p. 240-243 ^{o}C; IR (KBr): ν=3275, 3290 (2NH), 1690 cm^{-1} (CON), ^{1}H NMR (DMSO-d_6): δ = 3.25, 3.40 (2t, 2H each, CH_2 each); 6.25 (s, 2H, 2CH), 12.01, 12.20 ppm (2s, 2H each, 2NH each, exchangeable with D_2O); MS: *m/z* (%) = 387 (M^+ + 1, 9).

Anal. Calcd. for $C_{10}H_{10}N_{16}O_2$ (386): C, 31.09; H, 2.59; N, 58.03. Found: C, 30.82; H, 2.61; N, 57.82%.

4.28. Synthesis of 16*a-c*: G*eneral Procedure*

The respective of **15***a-c* (0.006 mol) was treated with 20 cm^3 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 **16***a-c*.

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

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

Anal. Calcd. for $C_8H_2N_{16}$ (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 (16*b*)

Yield: 1.72g (80.00%); m.p. 230-232 ^{o}C; ^{1}H NMR (DMSO-d_6): δ = 4.46, (s, 2H, CH_2), 6.25 ppm (s, 2H each, 2CH); MS: *m/z* (%) = 336 (M^+ , 27).

Anal. Calcd. for $C_9H_4N_{16}$ (336): C, 32.14; H, 1.19; N, 66.67. Found: C, 32.09; H, 1.14; N, 67.09%.

4.31. Bis {1,2,4-triazolo[4,3-*d*]tetrazolo [5,1-*f*]-1,2,4-triazin-3-yl} ethane (16*c*)

Yield: 1.76g (78.57%); m.p. 250-271 ^{o}C; IR (KBr): ν=1620 cm^{-1} (C=N), MS:

m/z (%) = 351 (M^{+}+1, 19).

Anal. Calcd. for $C_{10}H_6N_{16}$ (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/cm^3. Twofold dilution of the compounds were prepared (800, 400, …. 6.25 g/cm^3). The microorganism suspensions at 10^6 colony Formin Unit 1 cm^3 (*CFU*/cm^3) concentration were inoculate to the corresponding wells. Plates were incubated at 36^{o}C for 24 to 28h 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.

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