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RESEARCH ARTICLE

Synthesis and antimicrobial activity of some pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4]triazino[5,4-f] [1,2,4,5]tetrazinone derivatives

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Abstract

In the present study, Preparation of a novel series of 2-(2-heteroaroylhydrazono)-N'-(4-fluorophenyl)propanehydrazonoyl chloride (**4a-d**), which is used as a key intermediate in the synthesis of a novel series of pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4]triazino[5,4-f][1,2,4,5]tetrazin-11(8*H*)-one derivatives (**10a-d**), via its reaction with 5-amino-1-phenyl-6-thioxopyrazolo [3,4-d]pyrimidin-4-one (**5**) in dioxane in the presence of TEA under reflux followed by dehydrative cyclization in boiling pyridine was described. The structures of all the newly synthesized heterocyclic compounds were established by considering elemental analysis, spectral data and an alternative synthetic route. The antimicrobial activity of some selected products was evaluated and showed good results.

Introduction

In continuation of our studies dealing with the utility of hydrazonoyl halides for synthesis of various bridgehead nitrogen polyheterocycles [1-11], we wish to report herein a new facile synthesis of pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4]triazino[5,4-f][1,2,4,5]tetrazin-11(8*H*)-one ring system and its various functionalized derivatives that have not been reported hitherto. Our interest in exploring a new simple synthetic strategy for the latter ring system is due to the fact that literature search reveals that various derivatives of the two ring systems namely pyrazolopyrimidine and triazinotetrazine were reported to exhibit various biological activities. For example, some derivatives of the former ring system showed antibacterial, antifungal [11, 12], antiphlogistic,antitumor [13], herbicidal [14] and in vitro antiviral and antitumor activities [15-19]. Also, some derivatives of the latter ring exhibit as potent inhibitors targeting CYP1A1 activity [20].

In view of these findings, it was interesting to explore the synthesis of the title ring system from fused triazinotetrazine and pyrazolopyrimidine and explore the biological activities of some of its derivatives.

Result and Discussion

2-(2-Heteroaroylhydrazono)-N'-(4-fluorophenyl) propanehydrazonoyl chloride (**4a-d**) were prepared *via* condensation of N'-(4-fluorophenyl)-2-oxopropanehydrazonoyl chloride (**1)** with appropriate acid hydrazide (**2a-d**) in absolute ethanol as depicted in Scheme 1.

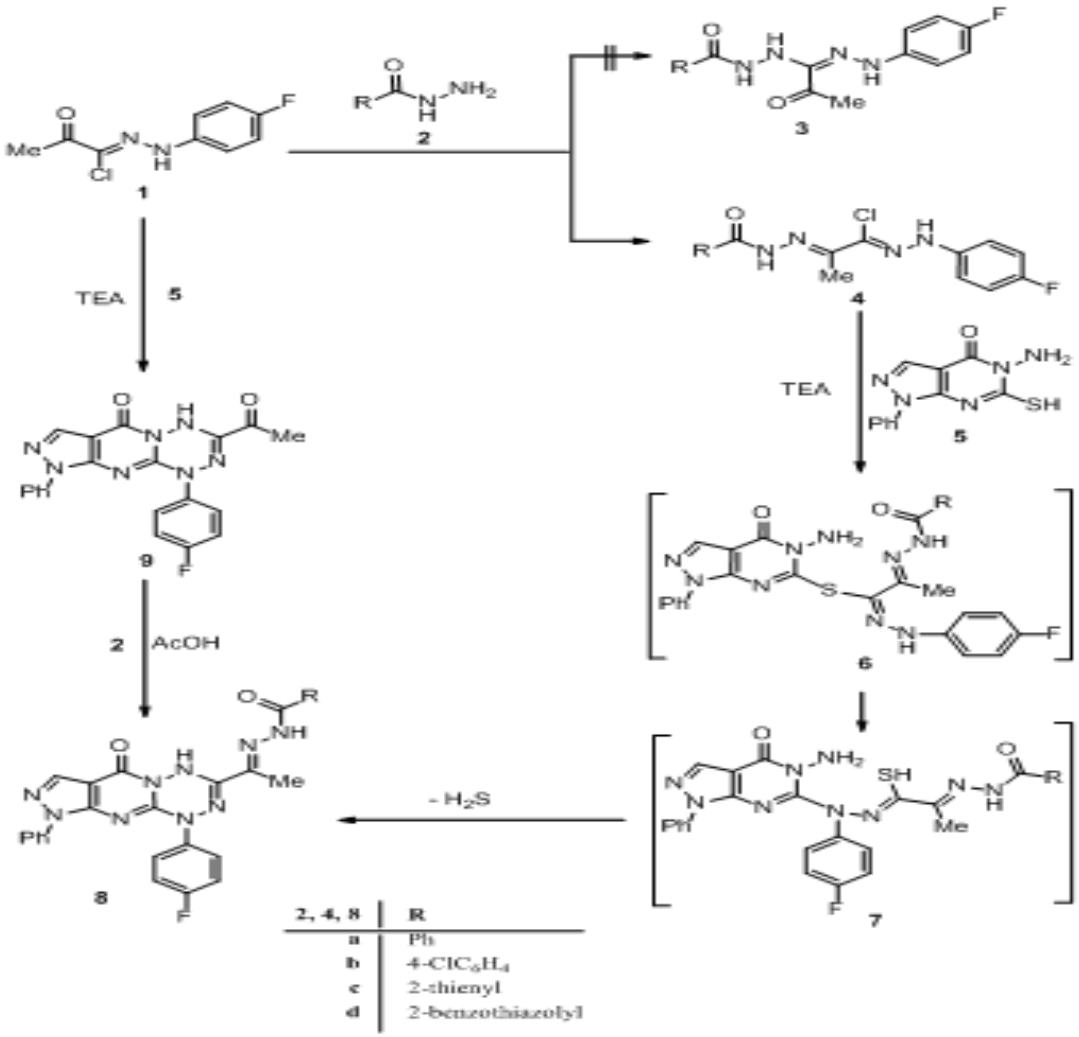
The structures of these compounds were confirmed from their spectral and micro analytical data. For example, the ^{1}H NMR spectrum of compound (**4a**) show signals at δ 2.36 (s, 3H, CH_3), 6.93-7.84 (m, 9H, Ar-H), 8.34 (s, br, 1H, NH), 12.63 (s, br, 1H, NH) ppm. The IR spectrum show different bands at 3316, 3268 (2NH), 1653 (C=O)cm^{-1}. The molecular ion peak of compound (**4a**) was observed at 332 (M^+) corresponding to the molecular formula $C_{16}H_{14}ClFN_4O$.

Treatment of the hydrazonoyl chlorides (**4a-d**) with 5-amino-1-phenyl-6-thioxopyrazolo[3,4-d]pyrimidin-

4-one **(5**) in dioxane in the presence of triethylamine at reflux until hydrogen sulfide gas ceased to evolve afforded the corresponding tetracyclic compounds **8a-d**. The isolated products were assigned the structure of pyrazolo[3',4':4,5]pyrimido[1,2-b] [1,2,4,5]tetrazin-3-yl)ethylidene)benzohydrazides **8a-d.** The structures of the compounds **8a-d** were established on the basis of spectroscopic and microanalyses data. For example, the IR of the compounds **8a** showed absorption bands at ν = 3312, 3120 (2NH), 1684, 1658 (2C=O) cm^{-1}, its ^1H NMR spectrum revealed signals at δ 2.49 (s, 3H, CH_3), 6.78-7.88 (m, 14H, ArH), 8.30 (s, 1H, pyrazole-CH), 9.30 (s, br, 1H, NH), 10.24 (s, br, 1H, NH) ppm. Moreover, they revealed the absence of the N-NH_2 proton signal present in the spectra of compounds **5** at δ 6.35. Its mass spectrum revealed a molecular ion peak at *m/z* 521.

A plausible mechanism to account for the formation of **8** is shown in Scheme 1.

It is suggested that, reactions of thione **5** with hydrazonoyl halides **4** presumably proceed through initial alkylation of **5** to give the thiohydrazonate esters **6**. This is followed by Smiles type rearrangement [20-23] of the latter esters to form the respective thiohydrazides **5**, which in turn underwent cyclization to give **8** as end products.



Scheme 1. Synthesis of pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4,5]tetrazin-3-yl)ethylidene)benzohydrazide (**8a-d**).

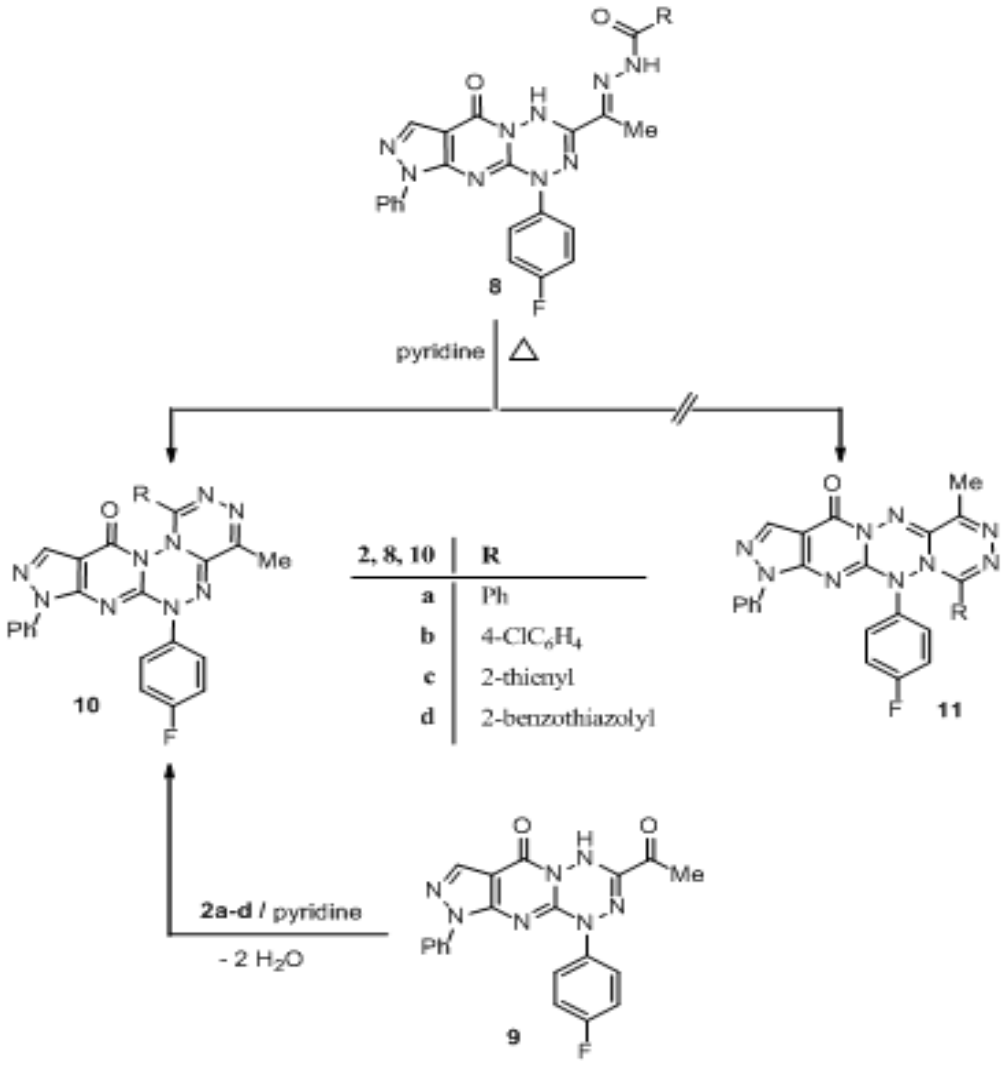
The structure of **8** was proved chemically *via* an alternative method (Scheme 1). Thus, reaction of compound **1** with **5** in dioxane in the presence of triethyl amine at refluxed to formation of compound **9**. Treatment of the latter with **2a-d** led to formation of product which is identical in all respects (mp, mixed mp and IR) with compound **8**.

The structure of compound **9** was substantiated by spectral (Mass, IR, and ^{1}H NMR) and elemental analyses data (See Experimental).

Reflux of compounds **8a-d** with dry pyridine afforded, *via* dehydrative cyclization, a product identified as pyrazolo[3',4':4,5]pyrimido[1,2-b] [1,2,4]triazino[5,4-f][1,2,4,5]tetrazin-11(8*H*)-one **10a-d** (Scheme2).

The structures of isolated products **10a-d** were evidenced by spectral data together with elemental analyses. For instance, the IR spectra of products display in each case the absorption no bands in the region of 3360-3150 and 1665-1690 cm^{-1} due to the (NH) and (C=O) groups, respectively. In ^{1}H NMR spectra all the products have no exchangeable singlet signals in the region of δ 9.25-11.00 ppm (D_2O exchangeable) assignable to the (NH) protons.

Finally, to provide a conclusive evidence for the assigned structure **10a-d**, we reacted compound **9** with **2a-d** under thermal conditions in pyridine to afford products that proved identical in all respects (IR, MS, mp. and mixed mp.) with **10a-d** (Scheme 2)**.**



Scheme 2. Synthesis of pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4]triazino[5,4-f][1,2,4,5]tetrazin-11(8*H*)-one derivatives (**10a-d**).

Antimicrobial Evaluation

The newly synthesized target compounds (**8a-d** and **10a-d**) were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (SA) and *Bacillis subtilis* (BS) as examples of Gram-positive bacteria and *Pseudomonas aeruginosa* (PA) and *Escherichia coli* (EC) as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *Aspergillus fumigatus* (AF), and *Candida albicans* (CA) fungal strains. The organisms were tested against the activity of solutions of concentrations (5 μg/mL) and using inhibition zone diameter (IZD) in mm as criterion for the antimicrobial activity (agar diffusion method). The fungicides *Clotrimazole* and the bactericides *Streptomycin* were used as references to evaluate the potency of the tested compounds under the same conditions. The results are depicted in Table 1.

The results depicted in Table 1 revealed that most of the tested compounds displayed variable inhibitory effects on the growth of the tested Gram-positive bacteria and Gram-negative bacteria strains and also against fungal strains. In general, most of the tested compounds revealed better activity against the Gram-positive bacteria rather than the Gram-negative bacteria, most of the tested compounds revealed better antibacterial activity rather than antifungal activity.

- Compounds **8a** exhibited almost no activity against Gram-positive bacteria and Gram-negative bacteria strains.
- Compound **10a** exhibited no activity against Gram-negative bacteria strains.
- Compound **8c** and **10c** exhibited almost no activity against fungal strains.
- Compounds **10d** exhibited high activity against Gram-positive bacteria and Gram-negative bacteria and fungal strains.
- The other tested compounds showed comparatively good activity against all the bacterial and fungal strains.

The good activity of **10d** is attributed to the presence of pharmacologically active benzothiazole at position 1 of the tetraheterocyclic ring system.

Table 1. Antibacterial and antifungal activities of the tested compounds (8a-d and 10a-d)

Sample no.	Minimum inhibitory concentration (μg / mL sample)					
	Gram-negative		Gram-positive		Fungi	
	E. Coli	*P. Aeruginosa*	*S. Aureus*	*B. Subtilis*	*C. Albicans*	*A. Fumigatus*
8a	NA	NA	NA	NA	15.5 ± 0.04	18.3 ± 0.06
8b	17.2 ± 0.14	18.3 ± 0.05	19.7 ± 0.05	22.1 ± 0.05	13.1 ± 0.05	17.3 ± 0.05
8c	19.2 ± .04	18.5 ± 0.14	22.5 ± 0.14	24.5 ± 0.14	NA	NA
8d	21.5 ± .06	22.2 ± 0.2	23.8 ± 0.01	27.7 ± 0.06	13.8 ± 0.03	19.6 ± 0.02
10a	NA	NA	13.1 ± 0.08	14.2 ± 0.08	13.1 ± 0.08	17.2 ± 0.08
10b	19.2 ± 0.07	21.6 ± 0.02	20.3 ± 0.03	24.2 ± 0.02	15.6 ± 0.02	18.5 ± 0.02
10c	18.2 ± 0.08	20.1 ± 0.02	21.2 ± 0.02	23.2 ± 0.02	NA	NA
10d	24.5 ± .14	24.0 ± 0.06	24.2 ± 0.06	28.4 ± 0.06	17.2 ± 0.06	23.4 ± 0.03
Streptomycin	25.6 ± .17	24.3 ± 0.04	25.1 ± 0.04	29.2 ± 0.04	--	--
Clotrimazole	--	--	--	--	18.3 ± 0.06	26.2 ± 0.04

NA: No activity, data are expressed in the form of mean ± SD. Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (5 μg/mL) concentration of tested samples.

Conclusions

In summary, efficient syntheses and characterization of new tetraheterocyclic system, named pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4]triazino[5,4-f][1,2,4,5]tetrazin-11(8*H*)-one derivatives have been reported.

Experimental Section

Melting points were measured on an Electrothermal IA 9000 series digital melting point apparatus. IR spectra were recorded in potassium bromide discs on Pye Unicam SP 3300 and Shimadzu FTIR 8101 PC infrared spectrophotometers. NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer operating at 300 MHz (^{1}H NMR) and run in deuterated dimethylsulfoxide (DMSO-*d*6). Chemical shifts were related to that of the solvent. Mass spectra were recorded on a Shimadzu GCeMS-QP1000 EX mass spectrometer at 70 eV. Elemental analyzes were measured by using a German made

Elementar vario LIII CHNS analyzer. Antimicrobial activity was evaluated by the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. 5-Amino-1-phenyl-6-thioxopyrazolo [3,4-d]pyrimidin-4-one[24] and N'-(4-fluorophenyl)-2-oxopropane hydrazonoyl chloride[25] were prepared as previously reported in the respective literature.

Synthesis of 2-(2-heteroaroylhydrazono)-N'-(4-fluorophenyl)propanehydrazonoyl chloride derivatives (4a-d).

A mixture of appropriate acid hydrazide (2 mmol) and N'-(4-fluorophenyl)-2-oxopropanehydrazonoyl chloride **2** (0.428 g, 2 mmol) in 30 ml absolute ethanol was refluxed for 4 h. The formed precipitate was isolated by filtration, washed with methanol, dried and recrystallized from appropriate solvent to give products **4a–d.**

2-(2-Benzoylhydrazono)-N'-(4-fluorophenyl) propanehydrazonoyl chloride (4a).

Yellow solid (80%); mp = 268-9°C; IR (KBr): *v* 3316, 3268 (2NH), 1653 (C=O), 1591 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*6): δ 2.36 (s, 3H, CH_3), 6.93-7.84 (m, 9H, Ar-H), 8.34 (s, br, 1H, NH), 12.63 (s, br, 1H, NH); MS *m/z* (%): 332 (M^+, 2), 296 (10), 161 (16), 105 (100), 77 (81). Anal. Calcd. for $C_{16}H_{14}ClFN_4O$ (332.76): C, 57.75; H, 4.24; N, 16.84. Found C, 57.68; H, 4.20; N, 16.59%.

2-(2-(4-Chlorobenzoyl)hydrazono)-N'-(4-fluorophenyl) propanehydrazonoyl chloride (4b).

Yellow solid (77%); mp = 283-5°C; IR (KBr): *v* 3428, 3192 (2NH), 1668 (C=O), 1600 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*6): δ 2.47 (s, 3H, CH_3), 7.04-8.26 (m, 8H, Ar-H), 8.40 (s, br, 1H, NH), 12.71 (s, br, 1H, NH); MS *m/z* (%): 367 (M^+, 4), 330 (19), 195 (11), 139 (100), 111 (75), 75 (72). Anal. Calcd. for $C_{16}H_{13}Cl_2FN_4O$ (367.21): C, 52.33; H, 3.57; N, 15.26. Found C, 52.23; H, 3.46; N, 15.02%.

N'-(4-Fluorophenyl)-2-(2-(thiophene-2-carbonyl) hydrazono)propanehydrazonoyl chloride (4c). Yellow solid (81%); mp = 254-6°C; IR (KBr): *v* 3364, 3225 (2NH), 1655 (C=O), 1598 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*6): δ 2.36 (s, 3H, CH_3), 6.93-7.84 (m, 7H, Ar-H), 8.34 (s, br, 1H, NH), 12.63 (s, br, 1H, NH); MS *m/z* (%): 340 (M^++1, 1), 338 (M^+, 3), 302 (7), 269 (2), 152 (3), 111 (100), 57 (30). Anal. Calcd. for $C_{14}H_{12}ClFN_4OS$ (338.79): C, 49.63; H, 3.57; N, 16.54. Found C, 49.76; H, 3.50; N, 16.43%.

2-(2-(Benzo[d]thiazole-2-carbonyl)hydrazono)-N'-(4-fluorophenyl)propanehydrazonoyl chloride (4d). Yellow solid (76%); mp = 248°C; IR (KBr): *v* 3311, 3268 (2NH), 1654 (C=O), 1591 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-*d*6): δ 2.51 (s, 3H, CH_3), 7.03-8.44 (m, 8H, Ar-H), 10.39 (s, br, 1H, NH), 13.32 (s, br, 1H, NH); MS *m/z* (%): 390 (M^++1, 4), 389 (M^+, 9), 353 (11), 218 (24), 162 (58), 135 (89), 110 (100), 83 (88). Anal. Calcd. for $C_{17}H_{13}ClFN_5OS$ (389.83): C, 52.38; H, 3.36; N, 17.96. Found C, 52.16; H, 3.28; N, 17.68%.

Synthesis of 3-acetyl-1-(4-flurophenyl)-9-phenyl-1,4-dihydropyrazolo[3,4-d]pyrimido[1,2-b] [1,2,4,5]tetrazin-6-one (9).

To a mixture of 5-amino-1-phenyl-6-thioxopyrazolo [3,4-d]pyrimidin-4-one **5** (2.59 g, 10 mmol) and N'-(4-fluorophenyl)-2-oxopropanehydrazonoyl chloride **1** (2.14 g, 10 mmol) in dioxane (40 mL), triethylamine (1.4 mL, 10 mmol) was added. The reaction mixture was refluxed till hydrogen sulfide ceased to evolve (10 h). The solvent was evaporated and the residue was treated with ice/HCl mixture. The solid product was collected, washed with water and crystallized from DMF to give **9**. Yellow solid, yield 84%, mp. 270 ^{0}C (DMF); IR (KBr): *v* 3128 (NH), 1718, 1678 (2C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.54 (s, 3H, $COCH_3$), 7.21-7.46 (m, 5H, ArH), 7.76 (d, *J* =7 Hz, 2H, ArH), 7.88 (d, *J* = 7 Hz, 2H, ArH), 8.36 (s, 1H, pyrazole-CH), 9.30 (s, br, 1H, NH); MS *m/z* (%): 420 (M^+ + 1, 21), 403 (M^+, 100), 377 (65), 218 (54),111 (36), 77 (58); Anal. Calcd. for $C_{20}H_{14}FN_7O_2$ (403.37): C, 59.55; H, 3.50; N, 24.31. Found: C, 59.50; H, 3.54; N, 24.23%.

Synthesis of pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4,5]tetrazin-3-yl)ethylidene)benzohydrazide derivatives (8a-d).

Method A

A mixture of appropriate **4a-d** (1 mmol) and **5** (0.259 g, 1 mmol) in dioxane (20 mL) containing triethylamine (0.1 g, 1 mmol) was refluxed for 6-8 h. (monitored by TLC). The formed precipitate was isolated by filtration, washed with methanol, dried and recrystallized from DMF to give products **8a–d.**

Method B

A mixture of **9** (0.403 g, 1 mmol) and **2a-d** (1 mmol) in AcOH (20 mL) was refluxed for 4-8 h. (monitored by TLC). The product started to separate out during the course of reaction. The solid product was filtered, washed with water, dried and recrystallized from DMF to give the respective products **8a-d** in 75-80% yield which are identical in all respects (mp., mixed mp. and IR) to those prepared from method A.

N'-(1-(1-(4-Fluorophenyl)-6-oxo-9-phenyl-1,4,6,9-tetrahydropyrazolo[3',4':4,5]pyrimido[1,2-b]

[1,2,4,5]tetrazin-3-yl)ethylidene)benzohydrazide (8a).
Brown solid (69%); mp = 288°C; IR (KBr): *v* 3312, 3120 (2NH), 1684, 1658 (2C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 6.78-7.88 (m, 14H, ArH), 8.30 (s, 1H, pyrazole-CH), 9.30 (s, br, 1H, NH), 10.24 (s, br, 1H, NH); MS *m/z* (%): 522 (M^+ + 1, 21), 521 (M^+, 10), 377 (100), 218 (54),111 (36), 77 (58). Anal. Calcd. for $C_{27}H_{20}FN_9O_2$ (521.51): C, 62.18; H, 3.87; N, 24.17. Found C, 62.11; H, 3.68; N, 24.10%.

4-Chloro-N'-(1-(1-(4-fluorophenyl)-6-oxo-9-phenyl -1,4,6,9-tetrahydropyrazolo[3',4':4,5]pyrimido [1,2-b][1,2,4,5]tetrazin-3-yl)ethylidene)benzo-hydrazide (8b).
Brown solid (64%); mp = 297°C; IR (KBr): *v* 3343, 3130 (2NH), 1690, 1662 (2C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 6.70-7.86 (m, 13H, ArH), 8.32 (s, 1H, pyrazole-CH), 9.37 (s, br, 1H, NH), 10.41 (s, br, 1H, NH); MS *m/z* (%): 558 (M^+ + 2, 4), 556 (M^+, 13), 377 (54), 218 (39),105 (100), 75 (68). Anal. Calcd. for $C_{27}H_{19}ClFN_9O_2$ (555.95): C, 58.33; H, 3.44; N, 22.67. Found C, 58.44; H, 3.40; N, 22.57%.

N'-(1-(1-(4-Fluorophenyl)-6-oxo-9-phenyl-1,4,6,9-tetrahydropyrazolo[3',4':4,5]pyrimido[1,2-b] [1,2,4,5]tetrazin-3-yl)ethylidene)thiophene-2-carbohydrazide (8c).
Yellow solid (68%); mp = 292-4°C; IR (KBr): *v* 3318, 3145 (2NH), 1676, 1660 (2C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 6.71-7.77 (m, 12H, ArH), 8.27 (s, 1H, pyrazole-CH), 9.32 (s, br, 1H, NH), 10.34 (s, br, 1H, NH); MS *m/z* (%): 528 (M^+ + 1, 3), 527 (M^+, 8), 343 (75), 218 (32),111 (100), 77 (76). Anal. Calcd. for $C_{25}H_{18}FN_9O_2S$ (527.53): C, 56.92; H, 3.44; N, 23.90. Found C, 56.91; H, 3.38; N, 23.76%.

N'-(1-(1-(4-Fluorophenyl)-6-oxo-9-phenyl-1,4,6,9-tetrahydropyrazolo[3',4':4,5]pyrimido[1,2-b] [1,2,4,5]tetrazin-3-yl)ethylidene)benzo[d]thiazole 2-carbohydrazide (8d).
Brown solid (75%); mp = 312-4°C; IR (KBr): *v* 3322, 3129 (2NH), 1678, 1668 (2C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 6.68-7.87 (m, 13H, ArH), 8.33 (s, 1H, pyrazole-CH), 9.25 (s, br, 1H, NH), 10.42 (s, br, 1H, NH); MS *m/z* (%): 579 (M^+ + 1, 9), 578 (M^+, 19), 333 (34), 186 (54),105 (68), 77 (100). Anal. Calcd. for $C_{28}H_{19}FN_{10}O_2S$ (578.58): C, 58.13; H, 3.31; N, 24.21. Found C, 58.11; H, 3.08; N, 24.08%.

Synthesis of pyrazolo[3',4':4,5]pyrimido[1,2-b] [1,2,4]triazino[5,4-f][1,2,4,5]tetrazin-11(8*H*)-one derivatives (10a-d).
Method A
Reflux **8a-d** (1 mmol) in pyridine (20 mL) for 10 h. After cooling, the solid product was filtered, washed with water, dried and recrystallized from DMF to give the respective products **10a-d** in 75-80% yield**.**
Method B
A mixture of **9** (0.403 g, 1 mmol) and **2a-d** (1 mmol) in pyridine (20 mL) was refluxed for 8-10 h. (monitored by TLC). The product started to separate out during the course of reaction. The solid product was filtered, washed with water, dried and recrystallized from DMF to give the respective products **10a-d** which are identical in all respects (mp., mixed mp. and IR) to those prepared from method A.

6-(4-Fluorophenyl)-4-methyl-1,8-diphenyl-6*H*-pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4]triazino [5,4-f][1,2,4,5]tetrazin-11(8*H*)-one (10a).
Yellow solid (72%); mp = 318°C; IR (KBr): *v* 1665 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 6.69-7.89 (m, 14H, ArH), 8.32 (s, 1H, pyrazole-CH). MS *m/z* (%): 504 (M^+ + 1, 5), 503 (M^+, 100), 377 (47), 111 (65), 77 (89). Anal. Calcd. for $C_{27}H_{18}FN_9O$ (503.49): C, 64.41; H, 3.60; N, 25.04. Found C, 64.36; H, 3.53; N, 25.01%.

1-(4-Chlorophenyl)-6-(4-fluorophenyl)-4-methyl-8-phenyl-6*H*-pyrazolo[3',4':4,5]pyrimido[1,2-b] [1,2,4]triazino[5,4-f][1,2,4,5]tetrazin-11(8*H*)-one (10b).
Yellow solid (65%); mp = 306-8°C; IR (KBr): *v* 1669 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3), 6.72-7.88 (m, 13H, ArH), 8.28 (s, 1H, pyrazole-CH); MS *m/z* (%): 539 (M^+ + 2, 20), 537 (M^+, 64), 218 (42),105 (100), 75 (35). Anal. Calcd. for $C_{27}H_{17}ClFN_9O$ (537.93): C, 60.28; H, 3.19; N, 23.43. Found C, 60.38; H, 3.11; N, 23.36%.

6-(4-Fluorophenyl)-4-methyl-8-phenyl-1-(thiophen -2-yl)-6*H*-pyrazolo[3',4':4,5]pyrimido[1,2-b][1,2,4] triazino[5,4-f][1,2,4,5]tetrazin-11(8*H*)-one (10c).
Yellow solid (65%); mp = 313-5°C; IR (KBr): *v* 1670 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 6.69-7.91 (m, 12H, ArH), 8.33 (s, 1H, pyrazole-CH); MS *m/z* (%): 509 (M^+, 32), 302 (54), 154 (100),111 (67), 77 (64). Anal. Calcd. for $C_{25}H_{16}FN_9OS$ (509.52): C, 58.93; H, 3.17; N, 24.74. Found C, 58.77; H, 3.06; N, 24.56%.

1-(Benzo[d]thiazol-2-yl)-6-(4-fluorophenyl)-4-methyl-8-phenyl-6*H*-pyrazolo[3',4':4,5]pyrimido

[1,2-b][1,2,4]triazino[5,4-f][1,2,4,5]tetrazin-11 (8*H*)-one (10d).

Yellow solid (70%); mp = 285-7°C; IR (KBr): *v* 1658 (C=O) cm^{-1}; ^{1}HNMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 6.72-7.96 (m, 13H, ArH), 8.26 (s, 1H, pyrazole-CH); MS *m/z* (%): 560 (M^+, 100), 333 (43), 232 (38), 141 (78), 57 (54). Anal. Calcd. for $C_{28}H_{17}ClFN_{10}OS$ (560.56): C, 59.99; H, 3.06; N, 24.99. Found C, 59.78; H, 3.02; N, 24.67%.

Antimicrobial activity test

Agar diffusion well method to determine the antimicrobial activity

The microorganism inoculums were uniformly spread using sterile cotton swab on a sterile Petri dish Malt extract agar (for fungi) and nutrient agar (for bacteria). One hundred µL of each sample was added to each well (10 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24-48 h at 37 °C (for bacteria) and at 28 °C (for fungi). After incubation, the microorganism's growth was observed. Inhibition of the bacterial and fungal growth were measured as IZD in mm. Tests were performed in triplicate [26].

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