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Synthesis and antimicrobial evaluation of novel 1,2,4-triazine derivatives

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ABSTRACT

5-(4-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazin-6(1H)-one 4 was synthesized. Treatment of the latter with $POCl_3$ afforded the chloro derivative 5. Hydrazinolysis of compound 5 with hydrazine hydrate in ethanol yielded 6-Hydrazino-5-(4-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazine 6. Condensation of the latter with appropriate aldehydes gave the corresponding 1,2,4-triazolo[4,3-f] [1,2,4]triazine derivatives 7a-c. However, treatment of the parent triazine 4 with phosphorous pentasulphide in pyridine afforded the corresponding thione derivative 8. Two novel series of compounds were synthesized from the thione derivative 9a-c and 12a,b. All the compounds were screened for their antimicrobial activity.

Key words: 1,2,4-triazin-6(1H)-one/ thione/ 1,2,4-triazolo[4,3-f][1,2,4]triazine/antimicrobial activity.

INTRODUCTION

Triazines were reported to possess versatile biological activities viz anticancer, antiviral, antibacterial and antifungal activities[1-6]. The introduction of proper side chains bearing sulphur atom was found to improve antimicrobial activity, also the formation of benzylidene derivatives play a major role in increasing the antibacterial activities of this class of compounds[7,8]. However, the condensed triazolotriazine recently was found to possess excellent antibacterial and antifungal activities[9-11]. Therefore, herein this study we report the synthesis of new derivatives of 1,2,4-triazine-6(1H)ones/thiones bearing a furyl moiety hoping for good antimicrobial activity.

MATERIAL AND METHODS

Chemistry

Experimental

Elemental analyses were performed on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA) at the Microanalytical Unit of Cairo University. Melting points were determined in open capillaries on an electrothermal LA 9000 Series (Electrothermal Engineering Ltd., Essex, UK) and are uncorrected. TLC chromatography was performed on precoated silica gel 60F 254 plates (Merck CO., Darmstadt, Germany). Infrared spectra were recorded on Pye Unicam SP 1000 IR spectrophotometer (Thermoelectron CO., Egelsbach, Germany). ^{1}HNMR spectra were recorded on Varian Gemini EM-300 MHz NMR spectrophotometer (Varian CO., Fort Collins, USA). DMSO-d_6 was used as solvent, TMS was used as internal standard and chemical shifts were measured in δ ppm. Mass spectra were recorded on Varian MAT 311-A 70 e.v. (Varian CO., Fort Collins, USA).

Compound 1-4 were synthesized according to reported procedure[12,13].

6-Hydrazino-5-(4-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazine 6:

A mixture of compound 4 (0.01 mol, 2.71g) and 99% hydrazine hydrate (0.012 mol) in absolute

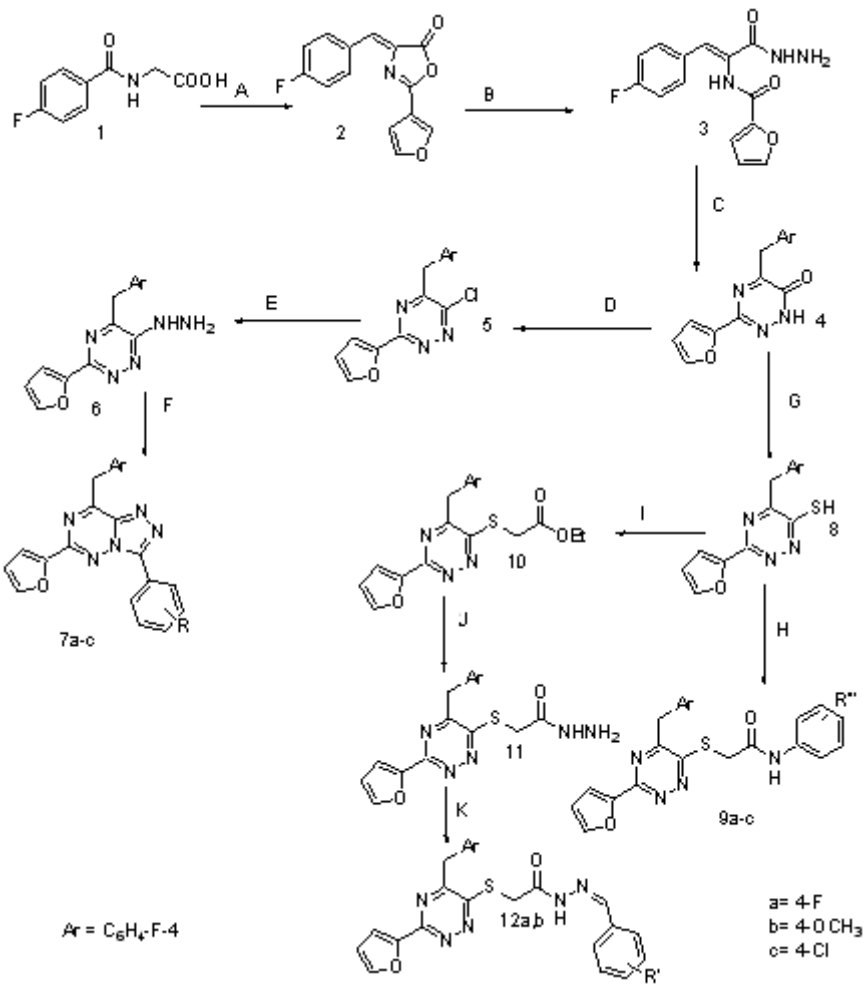
ethanol was heated at reflux for 6 h. Excess solvent was evaporated, residue washed with ether, recystallized from absolute ethanol to give the product.

General procedure for compounds 8-(4-fluorobenzyl)-3-(4-substitutedphenyl)-6-(furan-2-yl)-[1,2,4-triazolo[4,3-f][1,2,4]triazine 7a-c

A mixture of compound **6** (0.01 mol, 2.85 g) and appropriate aldehydes (0.01 mol), anhydrous potassium carbonate (0.3 g) was refluxed in DMF (20 ml) for 10 h. The reaction mixture was poured onto ice H_2O, the resulting residue was filtered dried and recystallized to give compounds 7a-c Table 1.

5-(4-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazin-6(1H)-thione 8

A mixture of compound **4** (0.01 mol) and P_2S_5 (2 g) in pyridine (10 mL) was heated at reflux for 10 h. The reaction mixture was poured onto ice H_2O/HCl, the resulting residue was filtered dried and recystallized from absolute ethanol to give compound 8.



Scheme 1: Synthesis of compounds 1-12: A, 2-furaldehyde, Ac_2/NaOAc/reflux; B, $N_2H_4H_2O$/EtOH/ reflux; C. 4% NaOH/reflux; D, $POCl_3$, water bath; E, $N_2H_4H_2O$/EtOH/reflux; F, appropriate aldyhydes/DMF/ anhydrous K_2CO_3/reflux, G, P_2S_5/pyridine/refulx; H. N-arylsubstituted chloroacetamides acetone/reflux/ J, $N_2H_4H_2O$/EtOH/stirring at room temperature; K, appropriate aldehydes/EtOH reflux

General procedure for preparation of compounds 9a-c

A mixture of 8 (0.01 mol, 2.87 g), the appropriate N-arylchloroacetamide (0.01 mol) and anhydrous sodium acetate (0.012 mol, 0.99 g) in absolute ethanol was refluxed for 6h. The mixture was cooled, the product was filtered, washed with water, dried, crystallized from the appropriate solvent. Table 1.

Ethyl 2-(5-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazin-6-ylthio)acetate 10

A mixture of compound 8 (0.01 mol, 2.87g) and ethylchloroacetate (0.01 mol), anhydrous potassium carbonate was refluxed in dry acetone (20 mL) for 24 h. The reaction mixture was poured onto ice H_2O, the resulting residue was filtered, washed with water, dried and recystallized from absolute ethanol to give our desired compound.

2-(5-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazin-6-ylthio)acetohydrazide 11

To suspension of 9 (0.01 mol) in absolute ethanol (20 mL), hydrazine hydrate (98%) (0.015 mol) was added and the mixture was stirred at room temperature for 24 h. The product was filtered, washed with water, dried and crystallized from ethanol.

2-(5-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazin-6-ylthio)-N-(4-substitutedbenzylidene) acetohydrazide 12 a,b

To suspension of 11 (0.02 mol, 1.79 g) in absolute ethanol (10 mL), the appropriate aldehyde (0.01 mol) was added and the mixture was refluxed for 3 h. Then the product was cooled, filtered, dried and crystallized, Table 1.

Table 1: Physical properties and molecular formula of the synthesized compounds

Cpd No.	m.p. (°C)	Solvent of Cryst.	% Yield	Mol. Formula M. Wt.	Calcd.	Found
7a	184-85	EtOH	60	$C_{21}H_{13}F_2N_5O$ 389.36	C 64.78	64.44
					H 3.37	3.47
					N 17.99	17.69
7b	210-12	EtOH	45	$C_{22}H_{16}FN_5O_2$ 401.39	C 65.83	65.38
					H 4.02	4.02
					N 17.45	17.51
7c	249-51	EtOH	60	$C_{21}H_{13}ClFN_5O$ 405.81	C 62.15	62.02
					H 3.23	3.96
					N 17.26	17.28
9a	140-43	EtOH	50	$C_{22}H_{16}F_2N_4O_2S$ 438.45	C 60.27	60.65
					H 3.68	3.92
					N 12.78	12.99
9b	159-61	EtOH	40	$C_{23}H_{19}FN_4O_3S$ 450.49	C 61.32	61.39
					H 4.25	4.54
					N 12.44	12.34
9c	180-82	EtOH/Ether	40	$C_{22}H_{16}Cl\ FN_4O_2S$ 454.9	C 58.09	58.54
					H 3.55	4.00
					N 12.32	12.34
12a	165-67	EtOH	80	$C_{23}H_{17}F_2N_5O_2S$ 465.48	C 59.35	59.99
					H 3.68	3.87
					N 15.05	14.80
12b	156-58	EtOH/Ether	65	$C_{24}H_{20}FN_5O_3S$ 477.51	C 60.37	60.69
					H 4.22	4.47
					N14.67	14.94

Table 2: Spectral data of the synthesized compounds

Comp. No	IR, (KBr, cm^{-1}), ^{1}HNMR (DMSO-d_6) and mass spectrum of the synthesized compounds
2.	M.S (m/z): 257[14.24 %], 123, [100 %], 95 [48.5], 75, [21.9 %] ,51 [16.9%].
3.	IR: 3320, 3160 (NH), 1669 (CO), 1632 (CO).
4.	IR : 3337(NH),2947 (aliphatic),1659 (CO), 1583 (CN). ^{1}HNMR (DMSO-d6): 3.66 (s, 2H,CH_2), 6.27 (s,1H, furyl-H), 6.59-6.6.62 (s, 2H, furyl-H), 7.34-7.37 (m, 2H, Ar-H), 7.80-7.85 (m, 2H, AAr-H), 9.38 (s, 1H, NH).; M.S (m/z): 271[100%], 242 [26.2%], 214 [10.8%], 121, [33.2%], 95 [26.2], 52 [3[22.2%].
5.	IR : 3075 (Ar-H), 2883 (aliphatic-H), 1606 (CN).
6.	IR : 3402, 3244 (NH,NH_2), 3070 (Ar-H), 1604 (C=N); ^{1}HNMR: 3.71 (s, 2H,CH_2), 6.06- 6.66 (m, 3H, furyl-H), 7.01-7.91 (m (m, 4H, Ar-H), 8.63 (s, 1H, NH), 9.02 (s, 2H, NH_2).
7a	IR: 3063 (Ar-H), 2923 (aliphatic-H), 1601 (CN). ^{1}HNMR : 3.63 (s, 2H, CH_2), 7.03-7.38 (m,5H, Ar-H), 7.79-7.97 (m, 4H, Ar-H), 8.71 (s, 2H, Ar-H). M.S (m/z): 389 [33.3%], 244 [47.9 %], 249 [100%], 175, [52.2%], 122 [58.2], 75 [32.2%]
7b	IR : 3040 (Ar-H), 2932 (aliphatic-H), 1618 (C=N). 1HNMR : 3.74 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 7.03-7.16 (m, 5H, Ar-H), 7.79-7.82 (m, 4H, Ar-H), 8.62 (s, 2H, Ar-H).
7c	IR : 3070 (Ar-H), 2933 (aliphatic-H), 1601 (C=N). 1HNMR : 3.64 (s, 2H, CH_2), 7.56-7.91 (m, 9H, Ar-H), 8.70 (s, 2H, Ar-H).
8	IR : 3402 (NH), 3077 (Ar-H), 2925 (aliphatic-H), 1600 (C=N).
9a	IR : 3281 (NH),1667 (C=O), 1606 (C=N). 1HNMR : 3.62 (s, 2H, CH_2), 3.71 (s, 2H, SCH_2), 7.13-7.58 (m,7H, Ar-H), 8.05-8.35 (m, 4H, Ar-H), 9.01 (s, 1H,NH).
9b	IR :3189 (NH),1665(CO), 1604 (C=N). 1HNMR : 3.74 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 4.01 (s, 2H, SCH_2), 7.08-7.47 (m, 11H, Ar-H), 10.02 (s, 1H, NH).
9c	IR : 3391 (NH), 1659 (CO), 1596 (C=N). M.S (m/z): 456 [0.2%, M+2], 454 [2 %, M+], 201 [15.1%],153 [20.3%] , 123 [100 %], 95 [69.2%], 75 [35.2 %].
10	IR : 1729 CO of ester, 3075 (Ar-H), 2979(aliphatic) ^{1}HNMR : 1.19-1.23 (t, 3H, CH_2), 3.72 (s, 2H, CH_2), 4.11-4.13 (q, 2H, CH_3), 3.96 (s, 2H, SCH_2), 7.39- 8.53 (m, 7H, Ar-H).
11	IR : 3400 (NH), 3199 (NH),1601 (CO)
12a	IR : 3378 (NH), 3079 (Ar-H), 1665(CO), 1594(C=N). M.S (m/z): 465 [25.9%], 244 [100%], 217 [22.9%], 121 [32.5%] 95 [59.2%], 44 [76.1%].
12b	IR :3218 (NH), 3075 (Ar-H), 1667(CO), 1606(C=N). 1HNMR : 3.69 (s, 2H, CH_2), 3.81 (s, 3H, OCH3), 3.94 (s, 2H, SCH_2), 7.02-7.37 (m, 5H, Ar-H), 7.78-7.87 (m, 6H, Ar-H), 8.60 (s, 1H, olefinic-H), 9.86 (s, 1H, NH). M.S (m/z): 477 [29%], 217 [100%], 185 [20.1%], 121 [39.3 %], 111 [74.2%], 95 [21%].

Antimicrobial activity

All the synthesized compounds were evaluated by the agar plate disc diffusion technique (14-16) . The test organisms were *Staphylococcus aureus* (NCTC-7447) as *Gram-positive* bacteria; *Escherichia* coli (NCTC-1041) as *Gram negative*, *Asperigellus flavus* used as a representative for multicellular fungi and *Candida albicans* was used as a representative for unicellular fungi. Sterilized filter paper discs (6mm) in diameter were wetted each with 10µL of a solution of the tested compounds containing 10mg/mLin DMF and the discs were then placed onto the surface of the agar plates (nutrient agar for bacteria and sabourauds dextrose agar for fungi) seeded with the test organism. Each plate contained 15 mL of the agar medium , previously seeded with 0.2 mL of 18 hours broth culture of each organism. The inoculated plates were incubated at 37°C for 48 hours and the inhibition zones were measured in mm. Discs impregnated with DMF were used as control. The antibacterial reference tetracycline and the antifugal reference duflucan discs were tested concurrently as a standard. The resulting inhibition zones are recorded in Table 3.

Table 3: Diameter of inhibition zones (mm) as criterion of antimicrobial activity of the synthesized compounds at concentration level of 5 mg/mL for both standard and tested compounds

Sample	*Escherichia coli*	*Staphylococcus aureus*	*Aspergillus flavus*	*Candida albicans*
7a	14	12	-	-
7b	-	-	-	-
7c	-	-	-	-
9a	15	16	-	-
9b	15	14	-	-
9c	14	13	-	-
12a	16	18	-	-
12b	16	16	-	-
St	34	33	17	20

Standard= tetracycline (antibacterial), diflucan (antifungal)

RESULTS AND DISCUSSION

Chemistry

p-Fluorohippuric acid 1 was prepared according to reported procedure[12], oxazolone of 2-furyl 2 was prepared by cyclocondensation using 2-furaldehyde and acetic anhydride, mass spectrum displayed a molecular ion peak at m/z 257 (84%)[13].

Hydrazinolysis of the latter in ethanol afforded N-(1-4-fluorophenyl)-3-hydrazinyl-3-oxoprop-1-en-2-yl)furan-2-carboxamide, the IR spectrum showed symmetrical stretching bands of NH, NH_2 at 3320,3160 cm^{-1} respectively 3.

Reflux of 3 in basic medium afforded the 5-(4-fluorobenzyl)-3-(furan-2-yl)-1,2,4- triazin-6(1H)-one 4. The ^{1}HNMR of 4 showed signals at δ 3.45 (s, 2H,CH_2), 6.27 (s,1H, furyl-H), 6.59-6.62 (s, 2H, furyl-H), 7.34-7.37 (m, 2H, Ar-H), 7.80-7.85 (m, 2H, Ar-H), 9.38 (s, 1H, NH); also mass spectrum displayed a molecular ion peak at m/z 271 (100%).

Phosphorylation of triazine using $POCl_3$ yielded the chloro derivative 5, reflux of the latter with hydrazine hydrate in ethanol afforded 6-Hydrazino-5-(4-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazine 6. The ^{1}HNMR spectrum of which in DMSO-d_6) showed bands corresponding to 8.63, 9.02 (NH, NH_2) which were cancelled by D_2O.

This key intermediate 6 was condensed with different aldehydes in DMF in presence of

anhydrous K_2CO_3 to give the corresponding cyclized products 8-(4-fluorobenzyl)-3-(4-substitutedphenyl)-6-(furan-2-yl)-[1,2,4-triazolo[4,3-f][1,2,4]triazine , mass spectrum of 7a showed a molecular ion peak at m/z 389 (33.3%) 7a-c**.**

Reaction of 4 with P_2S_5 in pyridine afforded the thione derivative 8. Reaction of 8 with N-aryl substituted chloroacetamides afforded the 2-(5-(4-fluorobenzyl)-3-(furan-2-yl)-1,2,4-triazin-6-ylthio)-N-(4-substitutedphenyl)acetamide 9a-c. The ^{1}HNMR of 9a in DMSO-d_6 displayes signals at δ3.62 (CH_2), 3.71 (SCH_2), 7.13-7.58 (Ar-H), 8.05-8.35 (Ar-H), 10.06 (NH). However, reaction of 8 with ethylchloroacetate in acetone in the presence of anhydrous k_2CO_3 cause formation of the corresponding thioether 10 .

The ^{1}HNMR of 10 showed signals at δ (DMSO-d_6): 1.19-1.23 (t,OCH_2), 3.72 (CH_2), 4.11-4.13 (q, CH_3), 3.96 (SCH_2).

The required hydrazide derivative 11 was prepared through the interaction of ester with hydrazine hydrate, condensation of the latter with proper aldehydes produced the target hydrazides 12a,b. ^{1}HNMR in DMSO-d_6 of 12b displayed signals corresponding to 3.81 (OCH_3), 3.94 (SCH_2), 9.86 (NH).

Antimicrobial activity

In vitro antimicrobial screening of the new compounds 7a-c, 9a-c and 12a,b using agar plate disc diffusion technique[14-16] revealed that compounds 9a, 9b, 12a and 12b showed good antibacterial activity with respect to E-coli and S.aureus relative to the standard tetracycline, however none of the compounds showed antifungal activity with respect to diflucan as a standard. It seems that presence of a fluoro group or a methoxy group have a good influence on the antibacterial activity, Table 3.

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