SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDY OF TRIAZOLO-PYRIMIDINE FUSED DERIVATIVES BASED ON 3-ACETYL COUMARIN

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Article History: **Received: 10.06.2023** **Revised: 28.06.2023** **Accepted: 18.07.2023**

Abstract

The3-(6-(5-substitutedfuran-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-4-yl)-2H-chromen-2-one1(a-e) reacted with hydrazine hydrate to form 3-(6-(5-substituted furan-2-yl)-2-hydrazinyl-1,4-dihydropyrimidin-4-yl)-2H-chromen-2-one 2(a-e). The compounds 2(a-e) then reacted respectively with formic acid and acetic anhydride yielded 3-(5-(5-substitutedfuran-2-yl)-7,8-dihydro-[1,2,4]triazolo[4,3-a]pyrimidin-7-yl)-2H chromen -2-one 3 (a-e) and 3-(5-(5-substituted furan-2-yl)-3-methyl-7,8-dihydro-[1,2,4]triazolo [4,3-a] pyrimidin-7-yl)-2H-chromen-2-one 4(a-e). All the Synthesized compounds were characterized by elemental analysis, ^{1}H NMR, IR and LC-MS spectral studies. Antibacterial activity of all the compounds was studied against gram positive and gram negative bacteria and antifungal activity of all the compounds was studied against various fungi.

Keywords: coumarin, triazolo-pyrimidine, spectral data studies, antibacterial activity and antifungal activity.

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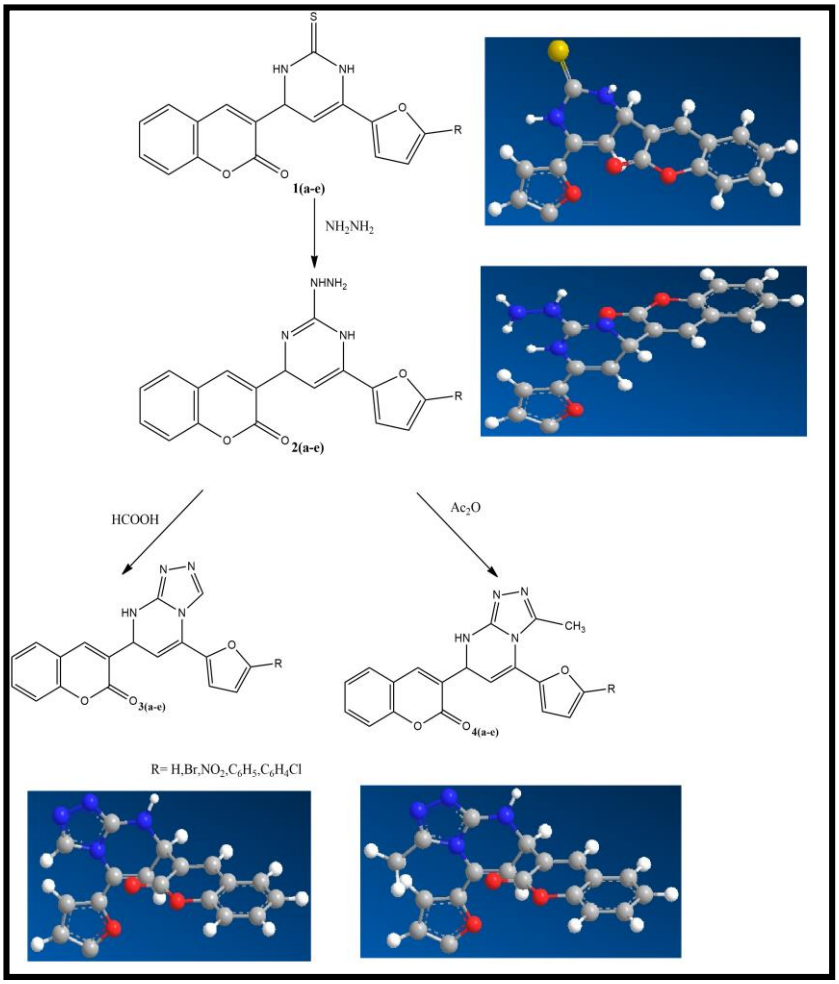
DOI: - 10.48047/ecb/2023.12.si10.00160

INTRODUCTION

In the field of Heterocyclic chemist's the triazolo-pyrimidine fused compounds are primary axis of biological activity. They are first investigated in 1909[1]. They are also found naturally [2, 3]. There derivatives are found application in both agricultural and medicinal chemistry [4, 5]. The triazolo-pyrimidine ring bear pharmaceutical activity like anticancer, antiparasitic, antifungal, antiviral and anti-inflammatory activities [6-10].

In our earlier communication, we reported the coumarine-pyrimidone derivatives [11]. In continuous of this we herewith present the work comprises the [Triazolo-pyrimidine] system with the background of biological properties of coumarine derivatives [12, 13] and review of triazolo-pyrimidine fused bio compounds. Thus, the approach of coumarine has been adopted and shown in Scheme-1.



EXPERIMENTAL

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer and ^{1}H NMR spectra were acquired at 400 MHz on a Bruker NMR spectrometer using DMSO–d6 as a solvent as well as TMS an internal reference standard. LC-MS of selected samples taken on LC-MSD-Trap-SL_01046. 3-(6-(5-substituted furan-2-yl)-2-thioxo-1,2,3,4-tetrahydr-opyrimidin-4-yl)-2H-chromen-2-one were prepared by reported in our earlier communication, we reported the coumarine-pyrimidone derivatives [11].

Synthesis of 3-(6-(5-substituted furan-2-yl)-2-hydrazinyl-1,4-dihydropyrimidin-4-yl)-2H-chromen-2-one 2(a-e)

A mixture of 3-(6-(5-substitutedfuran-2-yl)-2-thioxo- 1,2,3,4- tetrahydro pyrimidin-4-yl)-2H-chromen-2-one 1(a-e) (10mmol) and hydrazine hydrate(8mmol) in alcoholic KOH (2%,30ml) refluxed for 6hrs. It was then cooled and solid was isolated by filtration and recrystallized from ethyl acetate. The yields, melting points and other characterization data of these compounds are given in Table-1.

Table:-1 Analysis 2(a-e) derivatives

Compd.	Molecular formula Mol.wt.	LC-MS Data (m/z)	Yield %	M.P.* ^{0}C	Elemental Analysis			
					%C	% H	%N	%X X=Cl, Br
					Found Calcd.	Found Calcd.	Found Calcd.	Found Calcd.
2a	$C_{17}H_{14}N_4O_3$ 322	323.2	78	172	63.35 63.3	4.38 4.3	17.38 17.3	-
2b	$C_{17}H_{13}N_4O_3Br$ 401	401.8	73	166	50.89 50.8	3.27 3.2	13.96 13.9	19.92 19.9
2c	$C_{17}H_{13}N_5O_5$ 367	368.3	75	183	55.59 55.5	3.57 3.5	19.07 19.0	-
2d	$C_{23}H_{18}N_4O_3$ 398	398.9	72	174	69.34 69.3	4.55 4.5	14.06 14.0	-
2e	$C_{23}H_{17}N_4O_3Cl$ 432.5	433.7	76	172	63.82 63.8	3.96 3.9	12.94 12.9	8.19 8.1

***** Uncorrected

***Synthesis of* 3-(5-(5-substitutedfuran-2-yl)-7,8-dihydro-[1,2,4]triazolo[4,3-a] pyrimidin-7-yl)-2H-chromen-2-one 3(a-e)**

A solution of 3-(6-(5-substituted furan-2-yl)-2-hydrazinyl-1,4-dihydropyrimidin-4-yl)-2H-chromen-2-one 2(a-e) in formic acid (50ml) was refluxed for 6hrs. Vacuum distilled this reaction mixture to separated out solid precipitates, it was filtered, and washed thoroughly with cold water. The compound obtained was air dried and recrystallized from ethanol. The yields, melting points and other characterization data of these compounds are given in Table -2.

Table:-2 Analysis 3(a-e) derivatives

Compd.	Molecular formula (Mol.wt.)	LC-MS Data (m/z)	Yield %	M.P.* ^{0}C	Elemental Analysis			
					%C	% H	%N	%S
					Found Calcd.	Found Calcd.	Found Calcd.	Found Calcd.
3a	$C_{18}H_{12}N_4O_3$ 332	333.5	78	176	65.06 65.0	3.64 3.6	16.86 16.8	-
3b	$C_{18}H_{11}N_4O_3Br$ 411	412.2	73	167	52.57 52.5	2.70 2.6	13.62 13.6	19.43 19.4
3c	$C_{18}H_{11}N_5O_5$ 377	378.1	70	189	57.30 57.2	2.94 2.9	18.56 18.5	-
3d	$C_{24}H_{16}N_4O_3$ 408	409.4	72	177	70.58 70.5	3.95 3.9	13.72 13.7	-
3e	$C_{24}H_{15}N_4O_3Cl$ 442.5	443.7	76	173	65.09 65.0	3.41 3.4	12.65 12.6	8.01 8.0

***** Uncorrected

***Synthesis of* 3-(5-(5-substitutedfuran-2-yl)-7,8-dihydro-[1,2,4]triazolo[4,3-a] pyrimidin-7-yl)-2H-chromen-2-one 4(a-e)**

A solution of 3-(6-(5-substituted furan-2-yl)-2-hydrazinyl-1,4-dihydropyrimidin-4-yl)-2H-chromen -2-one 2(a-e) in acetic anhydride (50ml) was refluxed for 7hrs. Vacuum distilled this reaction mixture to separated out solid precipitates, it was filtered, and washed thoroughly with cold water. The compound obtained was air dried and recrystallized from ethanol. The yields, melting points and other characterization data of these compounds are given in Table-4.

Table:-3 Analysis 4(a-e) derivatives

Compd.	Molecular formula (Mol.wt.)	LC-MS Data (m/z)	Yield %	M.P.* ^{0}C	Elemental Analysis			
					%C	% H	%N	%S
					Found Calcd.	Found Calcd.	Found Calcd.	Found Calcd.
4a	$C_{19}H_{14}N_4O_3$ 346	346.8	76	173	65.89 65.8	4.07 4.0	16.18 16.1	-
4b	$C_{19}H_{13}N_4O_3Br$ 425	426.2	75	164	53.67 53.6	3.08 3.0	13.18 13.1	18.79 18.7
4c	$C_{19}H_{13}N_5O_5$ 391	392.4	68	186	58.31 58.3	3.35 3.3	17.90 17.8	-
4d	$C_{25}H_{18}N_4O_3$	422.8	72	170	71.08	4.29	13.26	-

	422				71.0	4.2	13.2	
4e	$C_{25}H_{17}N_4O_3Cl$ 456.5	457.3	79	177	65.72 65.7	3.75 3.7	12.26 12.2	7.76 7.7

***** Uncorrected

BIOLOGICAL SCREENING

Antibacterial activities

The antibacterial activities of all the compounds of three series were studied against gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*) and gram-negative bacteria (*E.coli, and klebsiella promioe*) at a concentration of 50µg/ML by agar cup plate method [14-16]. A methanol system was used as control in this method. Similar conditions using tetracycline as a control was used as standard for comparison. The area of inhibition of zone was measured in mm. Compounds 4e and 3e were found more toxic for microbes. Other compounds found to be less or moderate active shown in Tables -4.

Table:-3 Antibacterial Activity of Compounds 2(a-e), 3(a-e) and 4(a-e)

Compounds	**Gram +Ve**		**Gram –Ve**	
	Bacillus subtilis	***Staphylococcus aureus***	***Klebsiella promioe***	***E. coli***
2a	6	8	6	6
2b	9	13	11	11
2c	10	13	12	12
2d	7	9	8	10
2e	11	13	11	13
3a	9	11	9	10
3b	12	16	15	14
3c	13	16	14	14
3d	9	12	11	12
3e	13	16	15	15
4a	11	12	12	13
4b	14	17	18	17
4c	15	17	17	17
4d	11	13	14	15
4e	15	17	18	18
Tetracycline	13	15	15	14

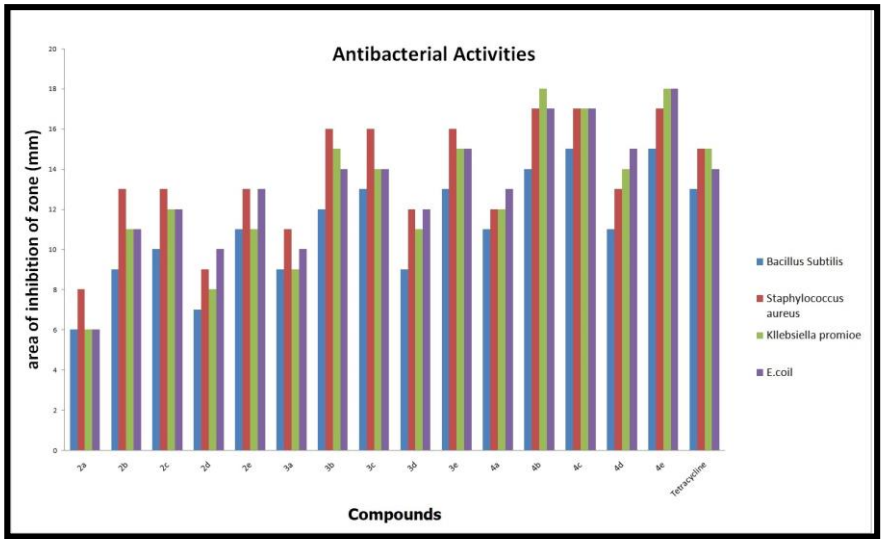


Fig-1. Histogram of antibacterial activity of Compounds 2(a-e), 3(a-e) and 4(a-e)

Antifungal Activities

The fungicidal activity of all the compounds of three series was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were *Aspergillus niger, Botrydepladia thiobromine, Nigrospora Sp, and Fusarium oxyporium*. The antifungal activities of all the compounds were measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200g, dextrose 20g, agar 20g and water 1c. Five days old cultures were employed. The compounds to be tested were

suspended (1000ppm) in a PDA medium and autoclaved at 120° C for 15 min. at 15atm. pressure. These media were poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. Flucanozol was used as standard. The percentage inhibition for fungi was calculated after five days using the formula given below:

Percentage of inhibition = 100(X-Y) / X

Where, X = Area of colony in control plate
Y = Area of colony in test plate

Compounds 4e and 3e were found more toxic for microbes. Other compounds found to be less or moderate active shown in Tables -5.

Table:-5 Antifungal Activity of Compounds 2(a-e), 3(a-e) and 4(a-e)

Zone of Inhibition at 1000 ppm (%)				
Compounds	***Aspergillus Niger***	***Botrydepladia Thiobromine***	***Nigrospora Sp.***	***Fusarium oxyporium***
2a	45	45	50	52
2b	58	51	62	61
2c	55	51	59	58
2d	52	48	57	57
2e	67	47	67	65
3a	45	48	50	51
3b	64	51	59	55
3c	66	53	65	62
3d	59	48	61	56
3e	68	56	67	64
4a	48	50	53	55
4b	67	53	62	59
4c	69	55	68	66
4d	62	50	64	60
4e	71	58	70	68
Flucanozol	95	93	94	92

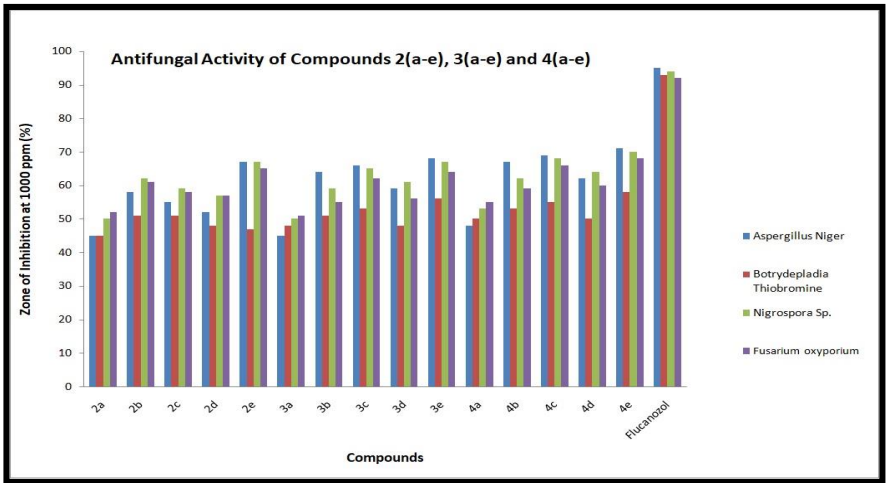


Fig-2. Histogram of antifungal activity of Compounds 2(a-e), 3(a-e) and 4(a-e)

RESULTS AND DISCUSSION

It was observed that 3-(6-(5-substituted furan-2-yl)-2-hydrazinyl-1,4-dihydropyrimidin-4-yl)-2H-chromen-2-one 2(a-e) have been synthesized by the reaction of 3-(6-(5-substitutedfuran-2-yl)-2-thioxo-1,2,3,4-tetrahydropyrimidin-4-yl)-2H-chromen-2-one 1(a-e) with hydrazine hydrate[11].

The structures of 2(a-e) were confirmed by elemental analysis and IR(KBr,cm^{-1}) spectra showing an absorption band at 3070-3025 (–C–H aromatic st.), 1680 (–C=N st.), 1600-1550cm^{-1} (conjugated C=C),1530 (–C=C– st.),1235(–N–N=C–st.),1670-1630 (C=O), 1120 (C-O-C), 3450 (-NH and $–NH_2$),1050(C-Cl), 1540, 1370 (NO_2), 590(C-Br).

^{1}H NMR (DMSO–*d*6,δ ppm): 6.50-6.75 (m, 2H, furan), 7.45–8.00 (m, 5H, Ar–H), 3.05 (d, 1H, –CH),6.20 (d,1H,=CH), 2.78,4.22 (s,2H,-NH) and 5.45(s,2H,-NH_2). The C, H, N analysis data of all compounds are presented in Table -1.

The structures of 3-(5-(5-substitutedfuran-2-yl)-7,8-dihydro-[1,2,4]triazolo[4,3-a]pyrimidin-7-yl)-2H-chromen-2-one 3(a-e) were confirmed by elemental analysis and IR(KBr,cm^{-1}) spectra

showing an absorption band at 3075-3025 (–C–H aromatic st.), 1680 (–C=N st.), 1600-1550 cm^{-1} (conjugated C=C),1525 (–C=C– st.),1235(–N–N=C–st.),1670-1635 (C=O), 1120 (C-O-C), 1050 (C-Cl), 1540, 1370 (NO_2),590(C-Br). ^1H NMR (DMSO–*d*6,δ ppm): 6.50-6.75 (m, 2H, furan), 7.45 –8.80 (m, 6H, Ar–H), 4.05 (d, 1H, –CH), 6.30 (d,1H,=CH) and 5.32 (s,1H,-NH).

The structures of 3-(5-(5-substituted furan-2-yl)-3-methyl-7,8-dihydro-[1,2,4] triazolo[4,3-a]pyrimidin -7-yl)-2H-chromen-2-one 4(a-e) were confirmed by elemental analysis and IR(KBr,cm^{-1}) spectra showing an absorption band at 3075-3020 (–C–H aromatic st.), 1680 (–C=N st.), 1600-1550 cm^{-1} (conjugated C=C),1525 (–C=C– st.),1235(–N–N=C–st.),1670-1635 (C=O), 1120 (C-O-C), 2820-2850 (-CH_3), 1050(C-Cl), 1540, 1370 (NO_2), 590(C-Br). ^1H NMR (DMSO–*d*6,δ ppm): 6.50-6.75 (m, 2H, furan), 7.45–8.80 (m, 5H, Ar–H), 4.05 (d, 1H, –CH),6.30(d,1H,=CH), 5.32 (s,1H,-NH) and 2.50(s,3H,-CH_3).

The examination of elemental analytical data reveals that the elemental contents are consistent with the predicted structure shown in Scheme-1. The IR data also direct for assignment of the predicted structure. The final structure of all compounds is confirmed by LC-MS. LC-MS data of all compounds are presented in Tables.

REFERENCES

1. C. Bülow, K. Haas, Ber. Dtsch. Chem. Ges. 1909, 42, 4638–4644.
2. M. El-Gendy, M. Shaaban, K. A. Shaaban, A. M. El-Bondkly, H. Laatsch, J. Antibiot. 2008, 61, 149–157.
3. E. H. Tee, T. Karoli, S. Ramu, J. X. Huang, M. S. Butler, M. A. Cooper, J. Nat. Prod. 2010, 73, 1940–1942.
4. F. S. Sabra, M. S. Mahmoud, Asian J. Agric Food Sci. 2015, 3, 103–108.
5. D. A. Pyatakov, A. N. Sokolov, A.V. Astakhov, A. Chernenko, A. Yu, A. N. Fakhrutdinov, V. B. Rybakov, V. V. Chernyshev, V. M. Chernyshev, J. Org. Chem. 2015, 80, 10694–10709.
6. A. M. Gamal-Eldeen, N. A. Hamdy, H. A. Abdel-Aziz, E. A. El-Hussieny, M. I. Fakher, Eur. J. Med. Chem. 2014, 77, 323–333.
7. M. A. H. Trianaa, M. H. Huynhb, M. F. Garavitoa, B. A. Foxc, D. J. Bzikc, V. B. Carruthers, M. Lofflerd, B. H. Zimmermanna, Mol. Biochem. Parasit. 2012, 184, 71–81.
8. Q. Chen, X. L. Zhu, L. L. Jiang, Z. M. Liu, G. F. Yang, Eur. J. Med. Chem. 2008, 43, 595–603.
9. S. Massari, G. Nannetti, J. Desantis, G. Muratore, S. Sabatini, G. Manfroni, B. Mercorelli, V. Cecchetti, G. Palu, G. Cruciani, A. Loregian, L. Goracci, O. Tabarrini, J. Med. Chem. 2015, 58, 3830–3842.
10. H. M. Ashour, O. G. Shaaban, O. H. Rizk, I. M. El-Ashmawy, Eur. J. Med. Chem. 2013, 62, 341–351.
11. R.A. Chauhan, H.S. Patel and S. Gulati, Eur. Chem. Bull., 12, 2065-2070 (2023).
12. V.F. Morales, A.P. Villasana-Ruiz, I. G. Veloz and S. G. Delgudo, Molecules, 28 (3), 2413 (2023).
13. M. Loncer, D. G. Sokae and M. Molnar, Czech. J. of Foo Sci.,(2023) https://doi.org./10.17221/178/2020-JFS.
14. M. A. Salam et. al., Conventional methods and future trends in antimicrobial susceptible testing, Saudi J. Biol. Sci., 30 (3), 103582 (2023).
15. P J Shah; H S Patel and B P Patel, Journal of Saudi Chemical Society, **2013**, 17, 307.
16. Standard Test method for Determinating the Antimicrobial activity of antimicrobial agents under Dynamic contact conditions, ASTM-E2149-20, USA (2020).