SYNTHESIS OF NOVEL HETEROCYCLES FROM 3-(3-ALKYL -5- MERCAPTO–1,2,4-TRIAZOLYLIMINOMETHYL) CHROMONES

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Abstract : 3- Formylchromone **1** reacts with 3- alkyl-4-amino-5-mercapto-1,2,4- triazole, **2a-2c** to give 3-(3-alkyl -5- mercapto–1,2,4-triazolyliminomethyl) chromones, **3a-3c**. On refluxing in nitrobenzene for 6 h, **3a-3c** afforded 2- (4-oxo-4H- [1] benzopyran-3-yl) [1] benzopyrano [3, 2-e] pyrimidin-5 (5H)- one **4** and 1- (4-oxo-4H- [1] benzopyran-3-yl) [1] benzopyrano [3,2-d] pyridazin-5 (5H)- one **5**. The compounds have been tested for their antibacterial and antifungal activities.

Key words: 3-(3-Alkyl -5- mercapto–1,2,4-triazolyliminomethyl) chromones, 3-formylchromone, 3-alkyl-4-amino-5-mercapto-1,2,4-triazole, pyranopyridazine, antibacterial and antifungal activities.

Introduction

Heterocyclic compounds having pyrimidine nucleus exhibit varied pharmacological activities (1-3). Other nitrogen hetrocycles such as pyridazines and triazoles show herbicidal (4,5) bactericidal (6), diuretic (7), antituberculosis (8) and anti-inflammatory (9) activities. Chromone derivatives such as benzopyranopyrimidine and pyranobenzopyrone show antiplatellet and cytotoxic activities (10,11). Various other chromone derivatives possess analgesic, anti-inflammatory (12) and antiallergic activities (13).

Thus, in view of the diverse biological activities exhibited by heterylchromones and in search of new biologically active compounds, we were prompted to undertake the synthesis of heterocycles from cheap and easily available 3-formylchromone. The present paper describes synthesis of novel benzopyranopyridazine and benzopyranopyrimidine from (4+2) cycloaddition reaction of 3-(3-alkyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones, 3a-3c in nitrobenzene. The compounds including 3a-3c have been screened for their antibacterial and antifungal activities.

Experimental

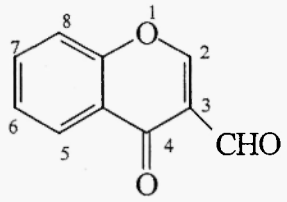
The melting points were taken in open capillaries and are uncorrected. The IR spectra were recorded on Perkin Elmer RXI spectrometer using KBr, ^{1}H NMR spectra on Bruker DRX-300 MHz in deuteriochloroform or hexamethyldeuteriodimethylsulfoxide with TMS as internal standard and mass spectra on Jeol Sx 102 (FAB). Physical and spectral data of the prepared compounds are given in Table I and II respectively. Compounds **1** and **2** were synthesized by reported methods (14,15).

3-(3-Alkyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones, 3a-3c.

A mixture of **1** (1g, 5.75 mmol) and **2a-2c** (0.747 g, 5.74 mmol) in dry benzene (10 ml) containing a catalytic amount of p-toluene sulfonic acid was refluxed on water bath for 16 h. The deposited solid, on cooling was filtered off and recrystallized from chloroform-benzene to afford **3a-3c**. The characteristic data for these compounds are given in Table-1.

Table-1 : Characteristic data of compounds 3a-3c and 5

Compound	M.P, °C	Yield %	Formula M.W.	Calculated/ Found % C	% H	% N
3a	210-12	65	$C_{13}H_{10}O_2N_4S$	54.54	3.52	19.57
			286	54.13	3.12	19.10
3b	240	62	$C_{14}H_{12}O_2N_4S$	55.98	4.03	18.65
			300	55.58	4.10	18.90
3c	225	61	$C_{15}H_{14}O_2N_4S$	57.26	4.48	17.82
			314	56.80	4.20	17.41
5	220-22	40	$C_{20}H_{10}O_4N_2$	70.17	2.94	8.18
			342	69.87	2.59	8.41



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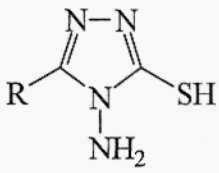

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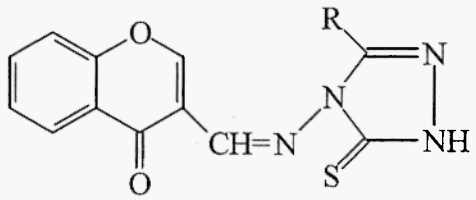
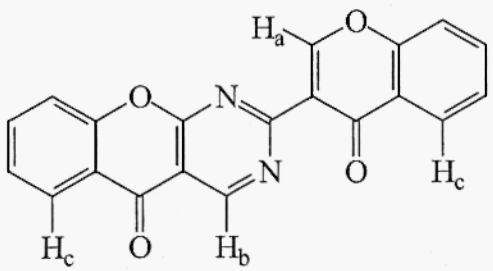
2a : R=CH_3
2b : R=C_2H_5
2c : R=C_3H_7



3a-3c



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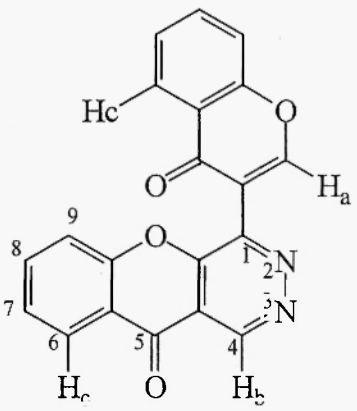

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2-(4-Oxo-4H-[1]benzopyran-3-yl) [1] benzopyrano[3, 2-e] pyrimidin-5(5H)-one, 4 and 1-(4-oxo-4H-[1] benzopyran-3-yl) [1] benzopyrano [3, 2-d] pyridazin-5(5 H)-one, 5.

The compound **3a-3c** (1 g, 3.50 mmol) was taken in nitrobenzene (10ml) and refluxed on an oil bath for 5 h. The solvent was removed under vacuum and the reaction mixture adsorbed on a column of silica gel. Elution of the column with benzene-ethylacetate (80:20, v/v) afforded **4** (16), 0.72 g, 60% and **5** (0.46g, 40%). See Table I for characteristic data of **5**.

Culture Media and Inoculum

Nutrient (N) and Sabouroud Dextrose (SD) (Hi-Media Pvt. Ltd., Mumbai, India) were used to culture the test bacteria and fungi respectively. The microbial cultures (test bacteria and *Candida albicans)* were grown at 37°C for 18 hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^5 CFU/ml. Similarly an inoculum of viable spore/mycelial fragments (10^5 CFU/ml) was prepared from filamentous fungi (17).

Antimicrobial Assays

Antimicrobial activity of the compounds was assayed by the disc diffusion method (18) with little modification. Briefly 0.1 ml of diluted inoculum (10^5 CFU/ml) of test organism was spread on nutrient agar/Sabouraud dextrose agar plates. Sterile paper disc impregnated with 50 μg of compounds and a disc without compound was used as a negative control. The plates were incubated for 18 h at 37 °C for test bacteria and *Candida albicans.* The fungi plates were incubated for 5-6 days at 28 °C. The antimicrobial activity was evaluated by measuring the zone of growth inhibition around disc of test organism. Antibiotics chloramphenicol (30μg/disc) and nystatin (100units/disc) (Hi-Media Pvt Ltd, Mumbai, India) were used as positive controls.

Results and Discussions

Chromones usually undergo ring opening reactions via nucleophilic attack at 2 position (19). However, due to presence of unsaturated functional groups at position 3, the reactivity of the system is changed and in certain cases undergo cyclo addition reactions (20,21). The intermediate adduct can be further transformed to other novel hererocycles which may not be easily synthesized by other routes (22).

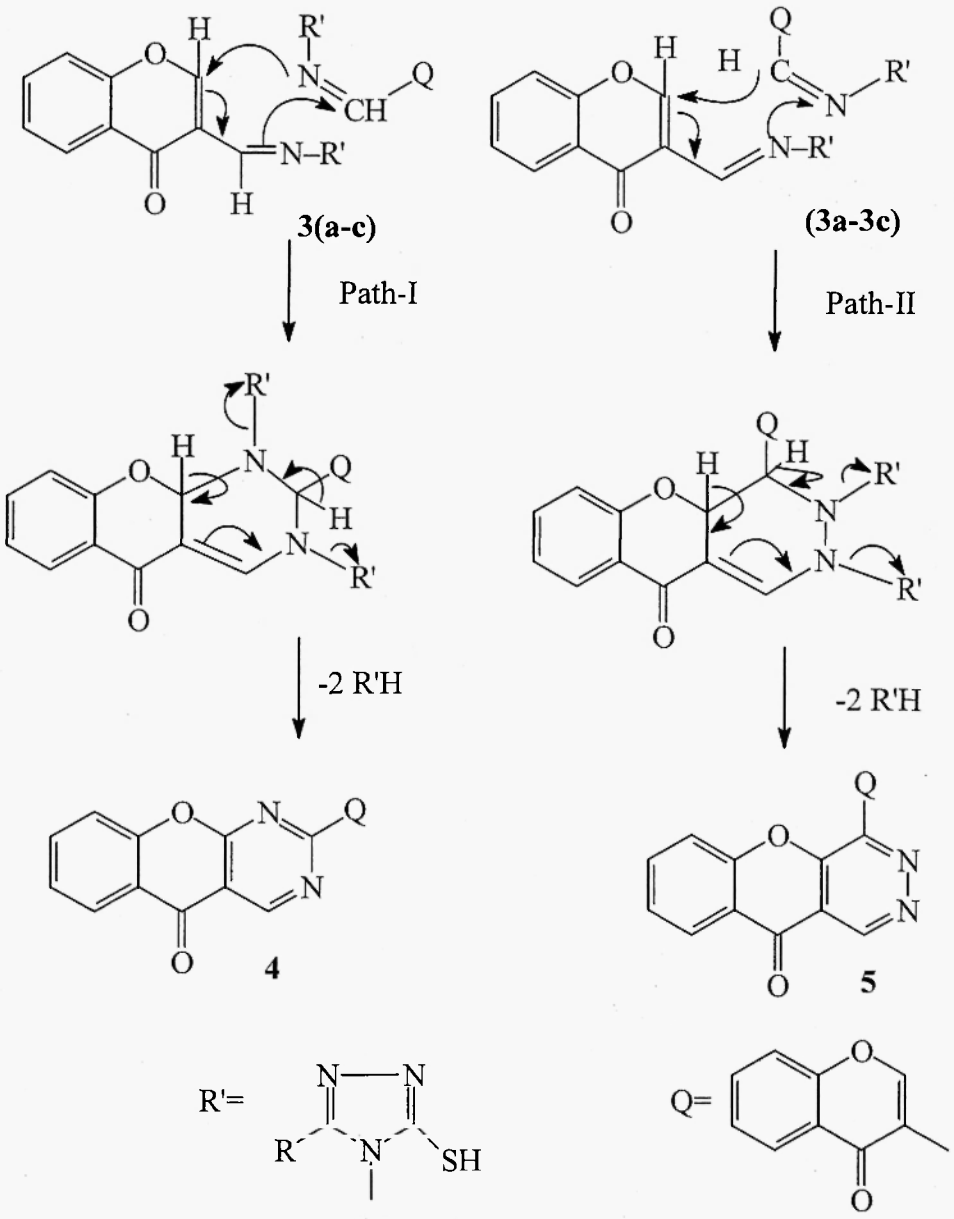
Thus, unsaturated chromone derivatives 3-(3-alkyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones, **3a-3c** were synthesized from 3-formylchromone by interaction with appropriate 1,2,4-triazoles, **2a-2c** in the presence of catalytic quantity of p-toluene sulphonic acid. The compounds, **3a-3c** were characterized on the basis of spectral data (Table-2). On refluxing in nitrobenzene for sometimes, **3a-3c** afforded compounds **4** and **5** in different ratio.

Table-2 : Spectral data of the prepared compounds 3a-3c, 4 and 5.

Compound	IR (ν,cm^{-1})	^{1}H NMR (δ, ppm)	MS (m/z, rel. %)
3a*	3424 (NH), 1649 (CO), 1284 (C=S).	2.37 s, 3H (CH_3); 7.57-7.93 m, 3H (Ar-H); 8.17 d, 1H (C-5); 9.13 s, 1H (C-2); 10.32 s, 1H (-CH=N-)	286 (M^+ 84), 172 (52), 165 (8), 138 (29), 123 (6), 120 (13), 114 (12).
3b	3451 (NH), 1655 (CO), 1231 (C=S).	1.33 t,3H (CH_3); 2.82 q, 2H (CH_2); 7.48-7.78 m, 3H (Ar-H); 8.33 d, 1H (C-5); 8.70 s, 1H (C-2); 10.37 s, 1H (-CH=N-); 10.46 br s, 1H (NH)	300(M^+100), 172(83), 165 (4), 138 (4), 123(2), 120 (8) 114 (4).
3c	3300 (NH), 1660 (CO), 1236 (C= S).	1.041 t, 3H (CH_3); 1.82 m, 2H (CH_2); 2.78 t, 2H (CH_2); 7.48-7.79 m, 3H (Ar-H); 8.30-8.33 dd, 1H (C-5); 8.71 s, 1H (C-2); 10.35 s, 1H (-CH=N-); 11.22 br s, 1H (NH)	314 (M^+90), 172 (100), 165 (12), 138(18), 123 (9), 120(12) 114 (12).
4*	1662 (CO)	7.48-7.84 m, 6H (Ar-H); 8.33 d, 1H (H_c); 8.42 d, 1H (H_c); 9.02 s, 1H (H_a); 9.69 s, 1H (H_b);	342 (M^+89),
5	1672 (CO)	7.26-7.63 m, 6H (Ar-H); 7.65, dd, 1H (H_c); 8.24 dd, 1H (H_c); 9.80 s, 1H (H_a); 10.30 s, 1H (H_b);	342 (M^+20), 313(6), 286(6),222(6) 172(3), 120(17), 116 (3)

* Measured in hexadeuteriodimethyl sulfoxide

Both the compounds **4** & **5** showed M^+ at m/z 342. Their IR spectra showed slightly broad bands at 1662 and 1670 cm^{-1} which are characteristic bands for chromone carbonyl groups. The ^1H NMR of **4** showed two doublets of H_c protons at δ 8.42, 8.33 and two sharp singlets of H_a, H_b protons at δ9.02, 9.69 respectively. The ^1H NMR of **5** showed signals for H_c and H_b protons at usual values but H_a proton appeared as slightly broad singlet at δ 9.80. The broadening of H_a singlet may be due to restricted rotation of chromone unit in **5** which is not noticed for the same proton in compound **4** because of more freedom to rotation of chromone unit. Since both **4** and **5** did not show any alkyl signals in their ^1H NMR spectra, we reasoned that when heated to high temperature, **3a-3c** had dimerized involving (4+2) cyclo addition reaction with expulsion of 3-alkyl-5-mercapto-1,2,4-triazole moiety (Scheme-1).



Scheme-1

All compounds were evaluated for their antibacterial and antifungal activities using DMSO as solvent. Interestingly only **3a-3c** exhibited pronounced broad-spectrum activity against both bacteria and fungi. The compound **3c** showed highest activity against *Staphylococcus aureus* (Gram positive) as well as *Salmonella typhimurium* (Gram negative) as compared to **3a and 3b**. However, **3c** was not active against *Pseudomonas aeruginosa*. Similarly **3a** showed relatively less activity to all test

bacteria except *Salmonella typhi*. The compound **3b** showed activity against all test bacteria. The zone inhibition size of these compounds is comparable with zone inhibition obtained by broad-spectrum antibiotic, chloramphenicol. Similarly, compound **3c** demonstrated highest activity against *C. albicans* and the activity is comparable with antifungal drug, nystatin. More interestingly the compounds **3c** and **3b** showed strong antifungal activity against filamentous fungi (*M. phaseolina, A. niger, F. solani* and *H. oryzae*) as compared to antifungal drug. However the antifungal activity of **3a** is at par with nystatin against *A. niger, F. solani* and *H. oryzae*. In general the antimicrobial activity was in order of **3c > 3b > 3a** (Table-3).

Table-3 : Antibacterial and antifungal activities of 3a-3c.

Organism	Test organisms	Inhibition zone size in mm			
		3a (50µg/ disc)	**3b** (50µg/ disc)	**3c** (50µg/ disc)	Antibiotic control*
Gram +ve Bacteria	**Staphylococcus aureus**	12	15	22	18
	Bacillus subtilis	08	14	15	22
Gram –ve Bacteria	*E. coli UP–2566*	09	12	12	21
	E. coli K12–J62	12	11	11	19
	Salmonella typhimurium MTCC98	10	12	16	19
	Salmonella typhi	--	14	14	20
	Pseudomonas aeruginosa	13	13	-	21
Yeast	**Candida albicans**	11	11	15	17
Filamentous fungi	Macrophomina phaseolina	31	37	37	21
	Aspergillus niger	14	31	29	18
	Fusarium solani	15	25	30	16
	Helminthosporum oryzae	15	22	21	16

*Antibiotic control: Chloramphenicol (30µg/ disc) for bacteria, Nystatin (100units/disc) for fungi and yeast.

Conclusions

The method offers an easy and one step synthesis of pyranopyrimidine **4** and pyranopyridazine **5** from 3-(3-alkyl-5-mercapto-1,2,4-triazolyliminomethyl) chromones, **3a-3c**. The antimicrobial evaluation of new compounds established that **3a, 3b** and **3c** showed interesting broad-spectrum activity against all test microorganisms with zone of growth inhibition range from 08-22 mm against test bacteria and 11-37 mm against test fungi. The activity was comparable to antifungal and antibacterial drugs i.e. nystatin and chloramphenicol respectively.

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