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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF NOVEL CHROMENO [4,3] PYRIMIDINE-2(5*H*)-THIONE ANALOGS

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Abstract:

*A series of novel chromeno [4,3] pyrimidine-2(5H)-thione derivatives were synthesized and characterized by FT-IR, ^{1}H-NMR, and Mass spectroscopy analysis with the aim of developing potent antimicrobial agents. The paper disc diffusion method and agar streak dilution method were performed for screening in vitro antimicrobial activity and the results are represented as a zone of inhibition and MIC, respectively. All compounds exhibited weak to potent anti-microbial activity against the tested microorganism such as B. subtilis, E. coli, A. niger & P. chrysogenum. The relationship between the functional group variation and the antimicrobial activity of the evaluated compounds was discussed. Out of various synthesized compounds, 3-(1-(dimethylamino)-2-(4-hydroxyphenyl) vinyl)-9-fluoro-5-hydroxy-3H-chromeno [4,3] pyrimidine-2(5H)-thione **(C1)** was found to be the most active compound.*

Keywords: *Pyrimidine, Chromene, Antimicrobial activity, Zone of inhibition, MIC*

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INTRODUCTION:

Bacteria are the most ancient forms of life on the planet, they are incredibly diverse and abundant. Bacteria are a group of microorganisms that are single-cellular with a transverse diameter of around one micron in size. Bacterial infections are among the most prevalent illnesses in the world, and they are regarded as one of the most dangerous difficulties in the medical sector in the past, present, and likely future [1]. Antibiotics, often known as anti-bacterial, are used to treat infections caused by bacteria. Antibiotic resistance has made it difficult to treat bacterial infections [2].

Pyrimidines and their annulated derivatives have a lot of biological activity and those are well-designed to process for chemical amendment. Sedative (barbiturates), antimetabolite (raltitrexed), diuretic drugs, and antiviral (idoxuridine, tenofovir, penciclovir), and all belong to this class of heterocyclic chemicals (triamterene) [3-4]. Despite decades of searching for bioactive drugs among molecules containing the pyrimidine group, their potential remains untapped [5-19].

On the other hand, in recent decades, the literature has been enriched with progressive findings about the synthesis and pharmacological activities of the chromene ring, which is a core structure in various synthetic pharmaceuticals displaying a wide variety of biological activities [20-24]. Based on the above-mentioned aspects, the synthetic drug moiety chromeno-[4,3]-pyrimidine-2(5*H*)-thione analogs were selected for this study. These scaffolds were synthesized by designing the scheme for synthesis and characterized using spectroscopic techniques such as FT-IR, ^{1}H-NMR, and GC-MS. Those compounds were screened for anti-microbial activity by exposure to various pathogens.

MATERIALS AND METHODS:

Chemistry

All solvents used were of laboratory grade and were obtained from SD fine chemicals (Mumbai, India), and Merck (Mumbai, India). Ciprofloxacin and Ketoconazole are received as gift samples from Dr. Reddy's laboratories, Hyderabad, India. Melting points were determined in open glass capillary tubes and are uncorrected. Compounds were routinely checked for purity on Silica gel G (Merck) Thin layer chromatography (TLC) plates; an iodine chamber and UV lamp were used to visualize TLC spots. The IR spectra were recorded in KBr pellets on (BIO-RAD FTS) FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on Bruker DPX-300 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization.

Synthesis of 9-fluoro-5-hydroxy-3*H*-chromeno [4,3] pyrimidine-2(5*H*)-thione (A):

In a dry round bottom flask (10 ml capacity) 0.001 mole 3-formylchromone and 0.002 mole thiourea were taken in dry ethanol. In this above mixture, one palette of KOH was added and the content was refluxed for 3 hours. The reaction was monitored with TLC. After completion of the reaction the content was cooled to room temperature and poured over crushed ice. The content was acidified with conc. HCl. The solid product obtained was separated by using filtration, washed with cold water. The product then dried and crystallized from acetic acid. IR (cm^{-1}): 3394 (OH), 3317 (NH), 3019 (Ar-CH), 1656 (C=N), 1604 (C=C), 1262 (C-F), 1150 (C=S), 1017 (C-O-C). ^{1}H-NMR (δ: ppm): 8.82 (s, 1H, C=S-NH), 7.51-7.88 (m, 3H, Ar-H), 6.68 (s, 1H, Chromene H-5), 5.39 (s, 1H, NH-CH=), 3.31 (s, 1H, OH). MS (EI) *m/z*: 250 (M^+). *Anal*. Calcd for $C_{11}H_7FN_2O_2S$.

Synthesis of 3-((dimethylamino)methyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3]pyrimidine-2(5*H*)-thione (B):

In the next step 0.001 mole of 9-fluoro-5-hydroxy-3,5-dihydro-2H-chromeno[4,3-d]pyrimidine-2-thione (**A**) react with 0.002 mole of formaldehyde and 0.001 mole of dimethyl amine leads Mannich reaction to get 3-((dimethylamino)methyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3] pyrimidine-2(5*H*)-thione (**B**). IR (cm^{-1}): 3287 (OH), 3012 (Ar-CH), 2970 (CH_3-CH), 1635 (C=N), 1602 (C=C), 1269 (C-F), 1178 (C=S), 1039 (C-O-C). ^{1}H-NMR (δ: ppm): 7.69-8.02 (m, 3H, Ar-H), 6.57 (s, 1H, Chromene H-5), 5.63 (s, 1H, N-CH=), 3.92 (s, 2H, CH_2 linkage), 3.40 (s, 1H, OH), 2.58 (s, 6H, $N(CH_3)_2$). MS (EI) *m/z*: 307 (M^+). *Anal*. Calcd for $C_{14}H_{14}FN_3O_2S$.

Synthesis of title compounds i.e., 3-(1-(dimethylamino)-2-(substituted phenyl)vinyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3]pyrimidine-2(5*H*)-thione (C1-C12):

Further compound (**B**) was reacted with substituted benzaldehyde (0.002 mol) in a beaker, to this sodium hydroxide solution 10 ml was added to make the solution alkaline, this was shaken and kept aside. The solid product separated out i.e., 3-(1-(dimethylamino)-2-(substituted phenyl)vinyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3]pyrimidine-

2(5*H*)-thione (**C1-C12**) was washed with water and recrystallized from absolute ethanol.

3-(1-(Dimethylamino)-2-(4-hydroxyphenyl)vinyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3]pyrimidine-2(5*H*)-thione (C1): IR (cm^{-1}): 3396 (OH), 3062 (Ar-CH), 2983 (CH_3-CH), 1672 (C=N), 1629 (C=C), 1247 (C-F), 1120 (C=S), 1032 (C-O-C). ^{1}H-NMR (δ: ppm): 7.09-7.83 (m, 7H, Ar-H), 6.39 (s, 1H, N-CH=), 6.11 (s, 1H, Chromene H-5), 5.42 (s, 1H, Ar-OH), 4.80 (s, 1H, =CH linkage), 3.42 (s, 1H, OH), 2.74 (s, 6H, $N(CH_3)_2$). MS (EI) *m/z*: 411 (M^+). *Anal*. Calcd for $C_{21}H_{18}FN_3O_3S$.

3-(1-(Dimethylamino)-2-(4-nitrophenyl)vinyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3]pyrimidine-2(5*H*)-thione (C6): IR (cm^{-1}): 3379 (OH), 3011 (Ar-CH), 2964 (CH_3-CH), 1644 (C=N), 1605 (C=C), 1539 & 1304 (NO_2), 1217 (C-F), 1106 (C=S), 1029 (C-O-C). ^{1}H-NMR (δ: ppm): 6.89-7.83 (m, 7H, Ar-H), 6.47 (s, 1H, N-CH=), 5.82 (s, 1H, Chromene H-5), 4.79 (s, 1H, =CH linkage), 3.20 (s, 1H, OH), 2.25 (s, 6H, $N(CH_3)_2$). MS (EI) *m/z*: 440 (M^+). *Anal*. Calcd for $C_{21}H_{17}FN_4O_4S$.

3-(2-(3-Chlorophenyl)-1-(dimethylamino)vinyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3]pyrimidine-2(5*H*)-thione (C10): IR (cm^{-1}): 3390 (OH), 3026 (Ar-CH), 2934 (CH_3-CH), 1659 (C=N), 1608 (C=C), 1206 (C-F), 1174 (C=S), 1032 (C-O-C), 759 (C-Cl). ^{1}H-NMR (δ: ppm): 7.14-7.82 (m, 7H, Ar-H), 6.38 (s, 1H, N-CH=), 5.99 (s, 1H, Chromene H-5), 4.35 (s, 1H, =CH linkage), 2.86 (s, 1H, OH), 1.89 (s, 6H, $N(CH_3)_2$). MS (EI) *m/z*: 431 (M^{+2}), 429 (M^+). *Anal*. Calcd for $C_{21}H_{17}ClFN_3O_2S$.

Biological activities

Test microorganisms:

The standard strains of microorganisms were procured from the National chemical laboratory, Pune, India, and the pathological strains were stored in the department of pharmaceutical biotechnology, MNR College of Pharmacy, Sangareddy, India. All the synthesized compounds were screened for antimicrobial activities by the paper disc diffusion technique. The antibacterial activity of the compounds was evaluated against one Gram-positive bacteria (*Bacillus subtilis* ATCC 6633) and one Gram-negative bacteria (*Escherichia coli* ATCC 25922). The antifungal activities of the synthesized compounds were evaluated against two fungi (*Aspergillus niger* ATCC 9029 and *Penicillium chrysogenum* ATCC 28089). Bacterial strains were cultured overnight at 37 °C in Mueller–Hinton broth and the yeast was cultured overnight at 30 °C in YEPDE agar for antibacterial and antifungal activity tests. Test strains were suspended in nutrient agar to give a final density of 5 X 10^{-5} cfu/ml.

Preliminary screening of antimicrobial activity (Paper disc diffusion method):

The sterilized (autoclaved at 120 °C for 30 min) medium (40-50 °C) was inoculated (1 ml / 100 ml of medium) with the suspension (10^5 cfu ml^{-1}) of the micro-organism (matched to McFarland barium sulphate standard) and poured into a petri dish to give a depth of 3-4 mm. The paper impregnated with the test compounds (100 µg ml^{-1} in dimethylformamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at RT and incubated at 37 °C for 24 and 48 h for anti-bacterial and anti-fungal activities, respectively. Ciprofloxacin (100 µg/disc) and Ketoconazole (100 µg/disc) were used as a standard for anti-bacterial and anti-fungal activities, respectively [25].

Determination of MIC (Agar streak dilution method):

MIC of the synthesized compound was determined by the agar streak dilution method. A stock solution of the synthesized compound (100 µg ml^{-1}) in dimethylformamide was prepared and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40-50 °C) containing the compound was poured into a petri dish to give a depth of 3-4 mm and allowed to solidify. Suspension of the microorganism was prepared to contain approximately 10^5 cfu ml^{-1} and applied to plates with serially diluted compounds in dimethylformamide to be tested and incubated at 37 °C for 24 h and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate [26].

RESULTS AND DISCUSSION:

Chemistry

In the present work, twelve novel chromeno[4,3]pyrimidine-2(5*H*)-thione derivatives **C1-C12** were prepared from 6-fluoro-4-oxo-4H-chromene-3-carbaldehyde & thiourea by multi-step synthesis. Initially, 9-Fluoro-5-hydroxy-3H-chromeno[4,3]pyrimidine-2(5H)-thione (**A**) was obtained by the reaction of 6-fluoro-4-oxo-4H-chromene-3-carbaldehyde with thiourea in presence of potassium hydroxide through cyclization reaction with the formation of the fused pyrimidine ring. In the next step, compound **A** undergoes Mannich

reaction by reacting with formaldehyde and dimethylamine and produced 3-((dimethylamino)methyl)-9-fluoro-5-hydroxy-3H-chromeno [4,3]pyrimidine-2(5H)-thione (**B**). Finally, compound **B** undergoes Schiff base reaction by reacting with various aromatic aldehyde and produced 3-(1-(dimethylamino)-2-(substitutedphenyl)vinyl)-9-fluoro-5-hydroxy-3H-chromeno[4,3]pyrimidine-2(5H)-thione (**C1-C12**). Throughout the reactions, TLC was performed to optimize the completion of reactions & their purity. The physicochemical properties of synthesized compounds are presented in Table 1.

Table 1: Physical parameters of the synthesized compounds (C1-C12)

Compound code	Yield (%)	M.P. (°C)	Molecular formula	Molecular weight
A	76	234-236	$C_{11}H_7FN_2O_2S$	250
B	79	210-212	$C_{14}H_{14}FN_3O_2S$	307
C1	73	130-131	$C_{21}H_{18}FN_3O_3S$	411
C2	70	155-156	$C_{22}H_{20}FN_3O_3S$	425
C3	79	168-170	$C_{22}H_{20}FN_3O_2S$	409
C4	75	141-143	$C_{21}H_{17}F_2N_3O_2S$	413
C5	72	185-187	$C_{21}H_{17}ClFN_3O_2S$	429
C6	77	172-174	$C_{21}H_{17}FN_4O_4S$	440
C7	74	197-199	$C_{21}H_{18}FN_3O_3S$	411
C8	78	144-145	$C_{22}H_{20}FN_3O_3S$	425
C9	73	166-168	$C_{22}H_{20}FN_3O_2S$	409
C10	75	179-181	$C_{21}H_{17}F_2N_3O_2S$	413
C11	71	149-150	$C_{21}H_{17}ClFN_3O_2S$	429
C12	70	136-148	$C_{21}H_{17}FN_4O_4S$	440

The appearance of absorption peak in IR at 3394 cm^{-1} & 3317 cm^{-1} corresponds to OH & NH stretching, respectively confirms the formation of 9-fluoro-5-hydroxy-3*H*-chromeno[4,3]pyrimidine-2(5*H*)-thione **(A)**. This is further supported by the presence of one proton singlet at δ 8.82 & 3.31 ppm corresponds to NH & OH proton, respectively. Likewise, the disappearance of absorption peak in IR around 3250 cm^{-1} corresponds to NH stretching and the disappearance of singlet around δ 8.50 ppm corresponds to one proton of NH in ^{1}H-NMR spectra approves the formation of Mannich base i.e., 3-((dimethylamino)methyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3] pyrimidine-2(5*H*)-thione (**B**). This is further supported by the appearance of two protons singlet for methylene linkage at δ 3.92 ppm. The disappearance of two protons singlet for methylene linkage around δ 4.00 ppm in ^{1}H-NMR spectra and appearance of peak around δ 6.50 ppm corresponds to one proton of =CH confirms the formation of 3-(1-(Dimethylamino)-2-(substitutedphenyl)vinyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3]pyrimidine-2(5*H*)-thione (**C1-C12**). The molecular weight & purity of prepared analogs were confirmed from their mass spectrum.

Biological activities

The zone of inhibition and MIC of title compounds were measured by the paper disc diffusion method and agar streak dilution technique, respectively. Ciprofloxacin and ketoconazole were used as standard drugs for comparing antibacterial and antifungal activity, respectively. The zone of inhibition and MIC of title compounds was compared effectively in Table 2 and 3, respectively. Antibacterial data clearly indicates that all tested analogs showed variable degrees of potency. In this research, overall it was found that title analog (**C1, C2 & C3**) exhibited good antimicrobial activity; title analogs (**C7, C8 & C9**) showed moderate antimicrobial activity; whereas all other title analogs (**C4, C5, C6, C10, C11, & C12**) produced only week antimicrobial activity. The presence of an activating group at the para position of the phenyl ring might be responsible for the good antimicrobial activity displayed by derivatives (**C1, C2 & C3**). In general, from this research, it was found that electron-donating groups containing title compounds displayed better antimicrobial activity than electron-withdrawing groups containing title compounds.

Table 2: Zone of inhibition of synthesized compounds (C1-C12)

COMPOUND CODE	ZONE OF INHIBITION (in mm)			
	BACTERIA		Fungi	
	B. subtilis	*E. coli*	*A. niger*	*P. chrysogenum*
C1	27	25	24	28
C2	25	25	26	24
C3	24	23	20	22
C4	13	10	11	14
C5	11	12	12	10
C6	12	13	10	12
C7	22	18	16	20
C8	19	21	17	18
C9	20	17	15	19
C10	7	6	9	6
C11	8	7	6	5
C12	6	7	5	5
Ciprofloxacin	32	29	ND	ND
Ketoconazole	ND	ND	26	33
Control	-	-	-	-

ND: Not determined; -: No growth

Table 3: MIC of synthesized compounds (C1-C12)

COMPOUND CODE	MIC (in µg/ml)			
	BACTERIA		Fungi	
	B. subtilis	*E. coli*	*A. niger*	*P. chrysogenum*
C1	6.25	3.13	6.25	6.25
C2	6.25	6.25	12.5	6.25
C3	6.25	12.5	6.25	12.5
C4	50	100	100	50
C5	100	50	100	100
C6	100	100	50	50
C7	12.5	12.5	25	12.5
C8	12.5	25	25	12.5
C9	25	25	50	25
C10	100	100	50	100
C11	100	100	100	100
C12	100	50	100	100
Ciprofloxacin	1.56	3.13	ND	ND
Ketoconazole	ND	ND	3.13	3.13

CONCLUSION:

Literature review reveals that pyrimidine and chromene have been reported to possess potent antimicrobial activities. Above observation prompted us to synthesize the 3-(1-(dimethylamino)-2-(substitutedphenyl)vinyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3] pyrimidine-2(5*H*)-thiones (**C1-C12**) with potent antimicrobial activities. Synthesized compounds were characterized by IR, ^{1}H-NMR, and mass spectral data. The spectral data of the synthesized compounds are in accordance with the assigned structures. Title analogs were tested for their anti-microbial activity by paper disc diffusion method and agar streak dilution method against *B. subtilis*, *E. coli*, *A. niger* & *P. chrysogenum*. All compounds exhibited weak to potent anti-microbial

activity compared to standard Ciprofloxacin and Ketoconazole. In general, from this research, it was found that electron-donating groups containing title compounds displayed better antimicrobial activity than electron-withdrawing groups containing title compounds. The most potent compound of the series was found to be 3-(1-(dimethylamino)-2-(4-hydroxyphenyl) vinyl)-9-fluoro-5-hydroxy-3*H*-chromeno[4,3] pyrimidine-2(5*H*)-thione (**C1**). Hence, this compound may serve as a clinically useful lead for antimicrobial drug development in the future.

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