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Synthesis, characterization and *in-vitro* anti-microbial and anti-cancer activity of novel fused benzo-chromene derivatives

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Abstract

An ecofriendly synthesis of *(E)* –N-(1-Substituted Phenyl)-3*H* –benzo[*f*]chromen-3 ylthioimidcarboxyacetohydrazides was carried out. The versatile use of benzo-chromenes has stimulated a considerable interest to explore the possible synthesis of novel fused compounds in which the benzo-chromene ring is fused with another biologically active molecule. An attempt was made to utilize this concept, that is the fusion of benzo-chromene with pyrimidin-4-one. Pyrimidin-4-one nucleus was chosen because it was found to possess various biological activities. The *(E)*-N-(1-SubstitutedPhenyl-3*H*benzo[*f*]chromenylthioimidcarboxyacetohydrazides were formed when the starting materials Substituted Benzaldehyde, Napthalene-2-ol and Ethyl cyano acetate were treated with Potassium Iodide, Iodine and Potassium Carbonate. The Ethyl-3-amino-1-substituted phenyl-1*H*-benzo[*f*]chromene-2-carboxylates were further treated with DMS, DMSO, CS_2, and finally with Hydrazine Hydrate to afford *(E)*–N-(1-Substituted Phenyl)-3*H*–benzo (*f*) chromen-3-ylthioimidcarboxyacetohydrazides. The novel Synthesized Compounds were characterized by M.P, IR, ^{1}HNMR and Mass Spectrum. These synthesized compounds were subjected to anti-bacterial studies using few Gram-Positive, Gram-negative and fungal organisms. The standard drug used for anti-microbial activity is Ampicillin and Ketoconazole respectively. Also is tested for their anti-cancer activity by MTT assay method. The tested Compounds exhibited significant anti-microbial and anti-cancer activity.

Key-Words: Benzo-chromenes, Pyrimidin-4-ones, Anti-microbial activity, Anti-Cancer Activity

Introduction

Fused benzo-chromenes continue to attract considerable attention because of their great importance, primarily due to a very wide spectrum of biological activities[1]. Benzo-chromenes and Pyrimidin-4-ones have a variety of pharmacological activities[2].

Therefore, it was thought of interest to utilize this concept for the synthesis of some novel fused benzo-chromene derivatives. The heterocyclic systems encompassing benzo-chromene and pyrimidin-4-one are explored to the maximum extent owing to their wide spectrum of pharmacological activities, such as anti-bacterial[3], anti-fungal[4], anti-cancer[5], anti-viral[6] and CNS stimulant[7] properties.

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Fused Benzo- chromenes are biologically active compounds with a wide spectrum of biological activities. Thus the synthesis of chromenes is of much importance to organic chemists and , in the view of the importance of the chromenes for diverse therapeutic activity, we considered it necessary to synthesize a high yielding, environmentally friendly and easy synthetic protocol for the synthesis of the substituted benzo-chromenes.all the synthesized compounds have been supported by their spectral data and screened for their anti-microbial & anti-cancer activity.

Material and Methods

All the chemicals were obtained from Aldrich chemical company (USA), Lancaster (USA) and S.D. Fine chemicals Limited (Mumbai). All the glassware is of borosilicate grade. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was ascertained by TLC on silica gel-G plate. Characterizations of synthesized compounds were done by spectral studies. Fourier transform infrared (FT-IR) spectra were taken in KBr on a

SHIMADZU spectrophotometer. ^{1}HNMR spectra were recorded on BRUKER AVANCE 300MHz spectrophotometer in $CdCl_3$ with TMS as internal standard. The chemical shift values are in delta (ppm). Mass spectra were recorded on Polaris Q apparatus (Thermo Electron) and the fragmentations were obtained by electronic impact (EI). The data is given as mass to charge ratio (m/z) and nominal masses were used for the calculation of molecular weights of the prepared products. Physical data, anti-microbial, anti-cancer activities of synthesized compounds were recorded in Tables.

General procedure

Step -1

Substituted Benzaldehyde (0.1moles), ethyl cyanoacetate (0.1moles) and β-naphthol (0.1moles) were stirred thoroughly in a beaker. Meanwhile I_2 (0.15gm), KI (0.1 gm) and K_2CO_3 (0.09 gm) were taken in another container and stirred until the iodine completely dissolves in it with some water added. Now both the mixtures were mixed together and the temp was increased to 100ºC. The reaction was stirred continuously at 100ºC for 2hr to obtain the solid yellowish color product. The completion of the reaction is known by TLC. It was then washed with $Na_2S_2O_3$ and filterd. The yield of the product is in between 72-78%. The melting points were in the range of 161-169^{0}C.

Step-2

Step 1 product (0.5moles) is taken with 50ml of DMSO & 0.5moles of carbon disulfide (CS_2) & mix with vigorous stirring. Stirring has to be continued for about 30min. To the above mixture add 10ml of 2M NaOH with vigorous stirring. After 15min, add 4.7ml of DMS drop wise. After addition, stirring was carried out for about 6hrs. The completion of the reaction is confirmed by TLC. After completion of reaction, mixture has to be poured into ice-water mixture. Then solid separates out and then filtered and recrystallized from ethanol.

Step-3

Step 2 product (0.5moles) is treated with hydrazine hydrate (99%, 0.1mole) in isoprpanol (10ml) heated under reflux on water bath until the methyl mercaptan evolution ceases. The solid is been obtained, washed and recrystallized with ethanol. The percentage yields were found to be in between 67-84%. The melting points were in the range of 176-193.

The melting points & yields were tabulated in the Table: 1.

Compound I

IR(KBr): 1595.6cm-^{1}(C=O), 1507cm^{-1}(C=N), 1669.0cm^{-1}(C=C), 3244.6cm^{-1}(NH-stretching), 2948.3cm^{-1}(C-H (aromatic), ^{1}HNMR($CDCl_3$): 4.6,s(-C-H), 2.0,s(-NH_2), 7.2-7.8, m(-Ar-H), 1.5, s(-SH). m/e(373.22m$^+$)(374.22m^{+1}) fragmentation ions(280,281,263,264,…).

Compound II

IR(KBr): 1603.7cm-^{1}(C=O), 1512.1cm^{-1}(C=N), 1662.0cm^{-1}(C=C), 3304.8cm^{-1}(NH-stretching),2926cm^{-1}(C-H (aromatic), 3304.0cm^{-1}(C-OH), ^{1}HNMR($CDCl_3$): 4.6,s(-C-H), 2.0,s(-NH_2), 7.2-7.8,m(-Ar-H), 1.5, s(-SH), 5.0,s(-OH).m/e(389.13m$^+$)(390.13m^{+1}) fragmentation ions(256,240,237,218,..).

Compound III

IR(KBr):1601.9cm-^{1}(C=O),1513.1cm^{-1}(C=N),1601.0cm^{-1}(C=C),3191.3cm^{-1}(NH-stretching),2926.9cm^{-1}(C-H aromatic), ^{1}HNMR($CDCl_3$): 4.6,s(-C-H), 2.0,s(-NH_2), 7.2-7.8,m(-Ar-H), 1.5, s(-SH). m/e(418m$^+$)(419^{+1}) fragmentation ions(380,352,318,…)

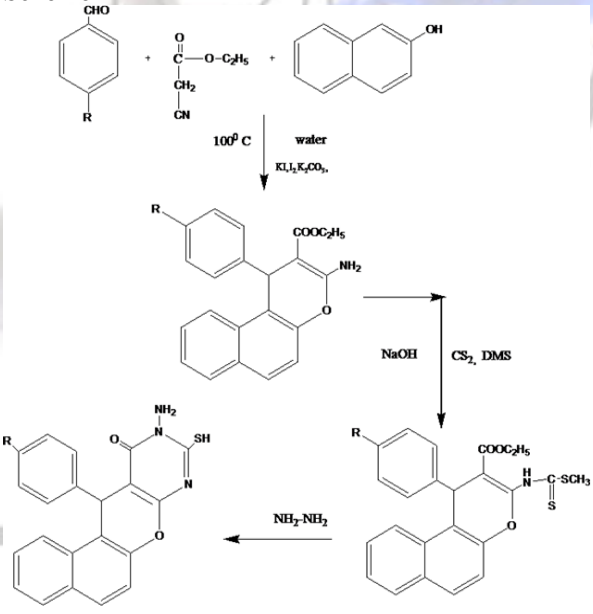
Compound IV

IR(KBr):1597.7cm-^{1}(C=O),1593.1cm^{-1}(C=N),1625.6cm^{-1}(C=C),3190.2cm^{-1}(NH-stretching),3065.2cm^{-1}(C-Haromatic), 618.2cm^{-1}(C-Cl), ^{1}HNMR($CDCl_3$): 4.6,s(-C-H), 2.0,s(-NH_2), 7.2-7.8,m(-Ar-H), 1.5, s(-SH). m/e(407.7m$^+$)(408.7m^{+1})(409.7m^{+2}) fragmentation ions(302,300,298,276,….).

Compound V

IR(KBr):1697.4cm-^{1}(C=O),1509.6cm^{-1}(C=N),1629.0cm^{-1}(C=C),3147.8cm^{-1}(NH-stretching),2967.8cm^{-1}(C-Haromatic), ^{1}HNMR($CdCl_3$): 4.6,s(-C-H), 2.0,s(-NH_2), 7.2-7.8,m(-Ar-H), 1.5, s(-SH), 2.9, s [-N(CH_3)$_2$]. m/e(415.41m$^+$)(416.41m^{+1}) fragmentation ions(298,246,232,…).

Scheme



***In vitro* anti-microbial activity[8-10]**

The synthesized compounds were reconstituted in Dimethyl formamide (DMF) as this does not demonstrate any anti-bacterial activity by itself. Initially, a suspension of nutrient agar medium was prepared. This suspension medium was inoculated by 100µl of *Staphylococcus aureus* ATCC9144 organism and the inoculation was possible only at 50°c. below this temperatyure the suspension of agar medium gets solidified and hence the uniform distribution of test organism cannot be achieved. Then immediately pour the inoculated media into the petridish under aseptic conditions. Maintenance of aseptic aseptic condition is an essential factor, which does not allow the contamination of other microorganism. Now the petridishes are kept aside for few minutes, in oreder to get solidified, forming a thin layer of about 2-4mm. sterile discs made of whatmann paper are used to apply the standard and test solutions on to the culture media. Initially the discs are stocked in control, standard drugs (Ampicillin & Ketoconazole) and test solutions separately and then, place on inoculated culture medium aseptically. Fine distance should be kept between discs. Petridishes are tightly packed and subjected to incubation at 37^0 C to 40^0 C for 48hr. Bacterial growth inhibition was determined as the diameter of inhibition zones around the disc. All tests were performed in triplicates. The resultant clear zones were measured in millimeters (mm) and compared against standard. Similar procedure is carried out for *Micrococcus luteus* ATCC11778, *Staphylococcus epidermidis* ATCC10987, *Klebsiella pneumonia* ATCC29212, *Escherichia coli mutant* ATCC25922 & *Candida albicans* ATCC2091 *Aspergillus niger* ATCC9029 respectively. The results were tabulated.

***In vitro* anti-cancer activity[11]**

MTT [(3-(4,5-dimethylthiazol-2yl)-2,5 diphenyltetrazoliumbromide] assay:0.1ml of the cell suspension (containing 5×10^6cells/100µl) and 0.1ml of the test solution (6.25µg - 100µg in 1% DMSO such that the final concentration of DMSO in media is less than 1%) were added to the 96 well plates and kept in 5% CO_2 incubator at 37^0 C for 72 hours. Blank contains only cell suspension and control wells contain 1% DMSO and cell suspension. After 72 hours, 20µl of MTT was added and kept in carbon dioxide incubator for 2 hours followed by propanol 100µl. The plate was covered with aluminum foil to protect it from light. Then the 96 well plates are kept in rotary shaker for 10-20 minutes.

After 10-20 minutes, the 96 well plates were processed on ELISA reader for absorption at 562nm. The readings were averaged and viability of the test samples were compared with DMSO control. The percentage growth inhibition was calculated.

The final results are in GI_{50} (growth inhibition 50), TGI (total growth inhibition), LC_{50} (lethal concentration 50). The cell lines used was HT29-Colon cancer, Hela-Cervical, HepG2-Liver cancer.

Results and Discussion

The synthesized compounds of the present study were characterized through IR , ^{1}H NMR & mass spectra. In the IR spectra of the*(E)* –N-(1-Substituted Phenyl)-3*H* –benzo[*f*]chromen-3-ylthioimidcarboxyacetohydrazides, the characteristic –NH_2 band appeared in the region of 3500-3220cm-1 and characteristic –SH absorption band in the region of 740-750cm-1, which was the most characteristic evidence of the cyclization.

In the ^{1}HNMR spectra of the *(E)* –N-(1-Substituted Phenyl)-3*H*–benzo[*f*]chromen-3-ylthioimidcarboxyacetohydrazides, the characteristic –NH_2 and SH peaks appeared in the region of 2.0 and 1.5 respectively. The absence of triplet at 1.3 to protons of -CH_2-CH_3 indicates the formation of cyclic structure and was the most characteristic evidence of the cyclization.

All the*(E)* –N-(1-Substituted Phenyl)-3*H* –benzo[*f*]chromen-3-ylthioimidcarboxyacetohydrazides of the present study showed expected characteristic molecular ion and fragmentation ion peaks.

The anti-microbial studies revealed that all the fused benzo-chromene derivatives showed significant anti-microbial activity when compared with that of standards ampicillin and ketoconazole. The zone of inhibition at 25, 50, 100µg/ml concentrations of synthesized compounds against Gram-positive, Gram-negative bacteria and fungi are measured by disc diffusion methods. The zone of inhibition of synthesized compounds was found to be similar to that of standards at 100µg/ml conc. Compounds containing III & IV (NO_2 & Cl) showed greater activity i.e, diameter(14-15 mm) where as compounds I(H), II(OH) & V($N(CH_3)_2$) diameter(10-12mm) showed mild to moderate activity. Results summarizes in tables 2-6 respectively.

Compound I & III shows significant anti-cancer activity in all cell lines but in toxicity wise compound I is lesser than III. Synthesized compounds II, IV and V were found to exhibit mild to moderate cytotoxicity in all cell lines.

Conclusion

In the present study novel fused benzochromene derivatives were synthesized. The benzo-chromene nucleus was fused with pyrimidine-4-one so that the therapeutic activity may be enhanced and was found to

be succeeded. The synthesized compounds were characterized by IR, ^{1}HNMR & Mass Spectrum. All the synthesized compounds showed characteristic absorption peaks in IR, ^{1}HNMR and Mass Spectrum. The synthesized novel fused benzo-chromene derivatives were subjected to *in-vitro* anti-microbial evaluation. The zone of inhibition at various concentrations of synthesized compounds against Gram-positive, Gram-negative bacteria and fungi are measured by disc diffusion methods.

The anti-bacterial and anti-fungal studies revealed that all the fused benzo-chromene derivatives showed significant anti-microbial activity when compared with that of standard. Compounds containing III & IV (NO_2& Cl) showed greater activity where as compounds I(H), II(OH) & V($N(CH_3)_2$)showed mild to moderate activity.

The cytotoxicity studies of the title compounds were evaluated against human cell lines (colon, cervical and liver).Title compounds were found to exhibit moderate cytotoxicity activities in all cell lines. Compounds I & III profoundly showed greater cytotoxic activity.

It was observed that compounds possesing electron withdrawing groups are showing greater activty. Therefore further studies are required for biologically more potent compounds in these series.

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Table 1: Physical data of synthesized compounds

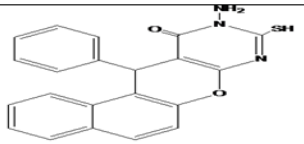
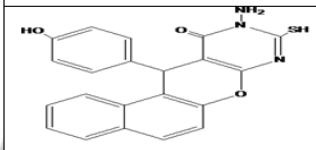
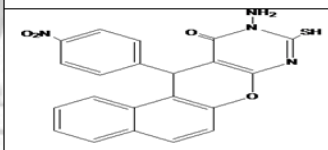
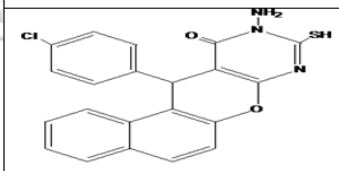
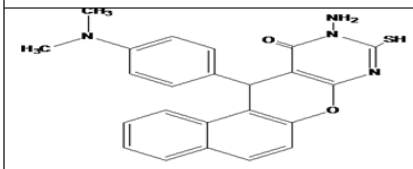
Structure	Molecular weight	Molecular formula	Melting point ^{O}C	% yield
	373.43	$C_{21}H_{15}N_3O_2S$	176-179	80.8%
	389.43	$C_{21}H_{15}N_3O_3S$	178-182	67.7%
	418.43	$C_{21}H_{14}N_4O_4S$	180-183	78.1%
	407.87	$C_{21}H_{14}ClN_3O_2S$	182-186	84.6%
	416.5	$C_{23}H_{20}N_4O_2S$	189-193	74.4%

Table 2: Zone of Inhibition of 25µg/ml Concentration of Synthesized Compounds against Gram (+) ve and Gram (-)ve Bacteria

S/No.	Compounds	Zone of inhibition in mm				
		Microorganisms				
		S. aureus ATCC9144	*S.epider-midis* ATCC10987	*M. luteus* ATCC11778	*K.pneumonia* ATCC29212	*E.coli mutant* ATCC25922
1.	I	4	5	5	4	5
2.	II	5	4	5	5	4
3.	III	7	6	6	7	6
4.	IV	6	7	7	6	8
5.	V	5	4	5	5	4
6.	Standard 10µg/ml	17	18	17	17	18
7	Control	-	-	-	-	-

Table 3: Zone of Inhibition of 50µg/ml Concentration of Synthesized Compounds against Gram (+) ve and Gram (-) ve Bacteria

S/No.	Compounds	Zone of inhibition in mm				
		Microorganisms				
		S. aureus ATCC9144	*S. epidermidis* ATCC10987	*M. luteus* ATCC11778	*K. pneumonia* ATCC29212	*E.coli mutant* ATCC25922
1.	I	11	11	10	10	10
2.	II	10	10	11	11	11
3.	III	12	12	13	12	13
4.	IV	13	12	13	12	11
5.	V	13	11	10	9	10
6.	Standard 10µg/ml	17	18	17	17	18
7	Control	-	-	-	-	-

Table 4: Zone of Inhibition of 100µg/ml Concentration of Synthesized Compounds against Gram (+) ve and Gram (-) ve Bacteria

S/No.	Compounds	Zone of inhibition in mm				
		Microorganisms				
		S. aureus ATCC9144	*S. epidermidis* ATCC10987	*M. luteus* ATCC11778	*K. pneumonia* ATCC29212	*E.coli mutant* ATCC25922
1.	I	10	11	12	10	12
2.	II	13	11	14	12	13
3.	III	14	15	14	15	14
4.	IV	15	14	14	15	14
5.	V	13	14	12	11	12
6.	Standard 10µg/ml	17	18	17	17	18
7.	Control	-	-	-	-	-

Table 5: Zone of Inhibition of Synthesized Compounds against *Candida albicans*

S/No.	Compounds	Zone of inhibition in mm		
		Concentration in µg/ml		
		25	50	100
1.	I	8	14	17
2.	II	7	12	16
3.	III	10	14	18
4.	IV	11	13	19
5.	V	8	12	17
6.	Standard 10µg/ml	16	18	18
7.	Control	-	-	-

Table 6: Zone of Inhibition of Synthesized Compounds against *Aspergillus niger*

S/No.	Compounds	Zone of inhibition in mm		
		Concentration in µg/ml		
		25	50	100
1.	I	7	13	16
2.	II	8	11	17
3.	III	9	14	19
4.	IV	9	14	18
5.	V	8	13	16
6.	Standard 10µg/ml	20	19	20
7.	Control	-	-	-

Table 7: Cytotoxicity Data of Synthesized Compounds

Sample	HT29			HepG2			Hela		
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}
I	18.3	29.5	68	18.4	43.8	81.0	29.7	63.7	›100
II	25.2	55.5	91.6	22.5	47.6	98.0	32.6	71.0	›100
III	22.8	49.5	›100	15.5	46.6	91.5	22.1	48.8	79.2
IV	25.2	58.5	98.7	27.7	48.0	97.7	31.3	75.5	›100
V	22.5	63.5	›100	41.6	61.9	›100	26.8	61.7	›100