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ANTIMICROBIAL AND ANTIINFLAMMATORY ACTIVITIES OF SOME NOVEL TRIAZOLOTHIADIAZOLES

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ABSTRACT

A novel series of 1,2,4-triazolo [3,4-b] [1,3,4] thiadiazoles were prepared by treating 4-amino -3-substituted -5-mercapto-1,2,4-triazoles with 2-amino-4-(substituted phenyl)-3-carboethoxy benzopyron. Their chemical structures were confirmed by means of spectral data IR, ^{1}H NMR and Elemental analysis. All the synthesized compounds were screened for antimicrobial activity and anti-inflammatory activities. Some of the synthesized compounds have shown very good antimicrobial and anti-inflammatory activities.

Key words: coumarin, sulfonation, antibacterial activity

INTRODUCTION

Combat against bacterial infections has resulted in the development of a wide variety of antibiotics. After years of misuse and overuse of antibiotics, bacteria are becoming antibiotic resistant, resulting in a potential global health crisis. There is already evidence that antibacterial resistance is associated with an increase in mortality. Frequently, it is recommended to use new antibacterial agents with enhanced broad spectrum potency. Therefore, recent efforts have been directed toward exploring novel antibacterial agents.

Substituted 1, 2, 4-triazoles and 1, 3, 4-thiadiazoles were reported to possess diverse biological applications including antimicrobial [1-3], analgesic [4], anti-inflammatory [5], anticonvulsant [6, 7], anticancer [8], antitubercular [9], antimalarial [10] and antiviral [11] activities. We thought to design a new series of triazolothiadiazole system by comprising of two heterocyclic systems namely coumarine and triazole system.

METHODOLOGY

All the chemicals used in the synthesis were obtained from standard commercial sources. Melting points were determined in open capillaries, using Boitus melting point apparatus, expressed in ^{o} C and are uncorrected. Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography. The ^{1}H NMR spectra of the compounds were recorded on Bruker AMX 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

General method of preparation acid hydrazides (1a): 4-Isobutyl phenyl propanic acid (0.1 mole) and ethanol (50ml) were taken with few drops of concentrated sulphuricacid and the mixture was refluxed for 6 hrs. The reaction mixture was concentrated by distilling off the excess of ethanol under reduced pressure and treated with a saturated solution of sodium bicarbonate. The ester obtained was used for the preparation of hydrazides directly.The ester (0.1mole) was dissolved in appropriate quantity of ethanol and the hydrazine hydrate 99% (0.1mole) was added. The reaction mixture was taken in round bottomed flask and refluxed for period of 10-18 hrs. Excess of ethanol

was distilled off under reduced pressure .It was then poured into ice cold water and the solid thus obtained was filtered. It was recrystallized from ethanol.

Preparation of potassium dithiocarbazinates (1b): Acid hydrazide (0.01 mole) was treated with carbon disulphide (0.01 mole) in the presence of alcoholic KOH (1.5 mole) and the reaction mixture was refluxed for a period of 3-4 hours. After cooling, the separated product was taken out, dried and used directly for the next step.

Preparation of 4-amino - 3 - substituted - 5 - mercapto - 1, 2, 4 -triazole (1c): A suspension of potassium dithiocarbazinate (0.1 mole), hydrazine hydrate (15 ml , 0.3 mole) and water (15 ml) was refluxed for 4-5 hrs until colour of the reaction mixture changed to green. Refluxing was continued for another 2 hrs when hydrogen sulphide gas evolution was stopped and homogeneous solution resulted. Dilution of the reaction mixture with water (50 ml) and subsequent acidification with dilute HCl gave a white precipitate. It was collected by filtration, washed with water and recrystallized from aq.ethanol.

Preparation of 2-amino-4-(substituted phenyl)-3-carboethoxy benzopyron: ($2a_1$-$2a_{11}$): Ethylcyanoacetate (0.1 mol), Appropriate Benzaldehyde (0.1 mol) and 2-naphthol (0.1 mol) in ethanol (80 ml) were treated with piperidine (1ml) and stirred at room temperature for 6-8 hour. Thus the solid separated was filtered, washed with ethanol and dried. The product was recrystallized from alcohol.

Synthesis of 1,2,4-triazolo [3,4-b] [1,3,4] thiadiazoles [$3a_1$-$3a_{11}$] : A mixture of $2a_1$ and 1c in equimolar quantities (0.01 mol) and phosphorous oxychloride (5-8 ml) was refluxed for 3-4 hrs, cooled and poured on crushed ice. The solid thus separated was filtered and washed with water and crystallized from alcohol. The purity of compound was established by single spot on TLC plates using silica gel G by using solvent system chloform:ethyl acetate (7:3).

Antibacterial activity: All the compounds synthesized in the present investigation were screened for their anti-bacterial activity by disc-diffusion method. Antibacterial activities were tested on nutrient medium against *Bacillus Pumilus*, *Bacillus subtilis*, *Escheria coli* and *Pseudomonas aeruginosa* which are representative types of gram positive and gram negative organisms respectively.

Anti-inflammatory activity: Anti-inflammatory activity of all synthesized compounds is carried out By Carrageenan induced rat paw oedema model. Six groups of albino rats of either sex (each comprising of six animals) weighing between 160-200 gms were deprived of food and water for 18 hours prior to the experiment. The standard diclofenac sodium and all synthesized compounds were administered orally to all rats. After 30 minutes 0.1 ml of 1% carrageenan suspension in normal saline was injected in to the sub plantar region of the hind paw of each rat. The oedema volumes of the injected paws were measured at 1st, 2nd and 4th hour. The difference between the paw volumes of treated animals were compared with that of the control group and the mean oedema volume was calculated.

From the data obtained mean volume of oedema, ± SEM and percentage reduction in oedema were calculated. Percentage reduction or inhibition in edema volume was calculated by using the formula.

$$\text{Percentage reduction} = \frac{Vo - Vt}{Vo} \times 100$$

Where, V_0 = Volume of the paw of control at time t

V_t = Volume of the paw of drug treated at time t.

From the data obtained the mean oedema volume and percentage reduction in oedema was calculated.

RESULTS AND DISCUSSION

All the compounds synthesized in the present investigation were characterized by TLC, IR and NMR data. Compound $3a_5$: IR (SPECTRA): 3430(NH stretching), 3090, 2965(aromatic CH stretching), 2937, 2927 (CH stretching of CH_3 and CH_2), 1224(NH bending). ^{1}HNMR δ(ppm): 0.85-.089(6 H, $(CH_3)_2$), 1.7(3H, CH_3 of CH-CH_3), 1.84-1.87 (1 H,CH of [CH-$(CH_3)_2$], 2.40-2.44(3H of CH_3),3.75(3H of OCH_3), 4.30-4.34(1H,CH of CH-CH_3) , 5.0(1H of Coumarin H), 6.6-6.8(2H of NH_2),7.0-8.2(14H of Ar-H).

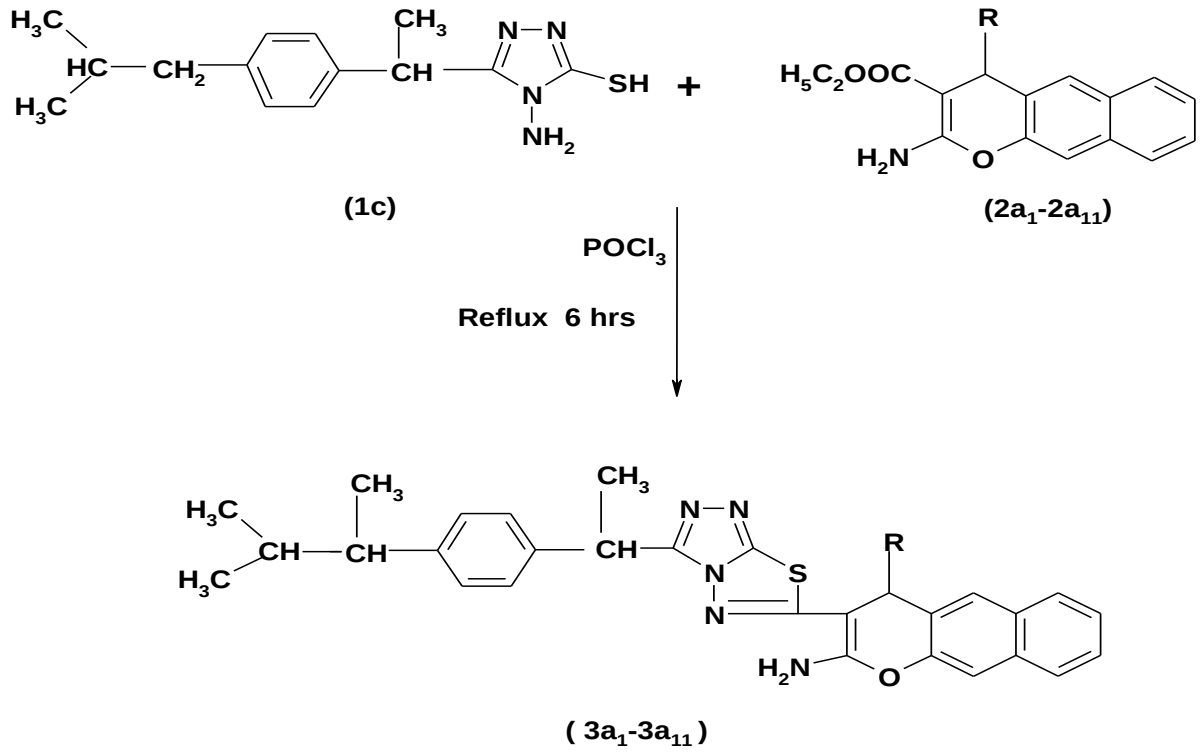
Antibacterial activity: All the synthesized compounds **$3a_1$-a_{11}** were screened for their antibacterial activity. From the anti-bacterial activity it was found that some compounds showed moderate and some are showed poor anti-bacterial activity when compared with standard employed for comparison against both Gram positive and Gram negative bacteria. Six compounds (**$3a_3$, $3a_4$, $3a_5$, $3a_6$, $3a_7$, $3a_8$**) at dose each of 200 mg/kg exhibited maximum inhibition.

Anti-inflammatory activity: The derivatives of triazolo thiadiazole derivatives of Ibuprofen series

were synthesized and selected eight compounds of this series were taken for the anti-inflammatory screening. The excellent anti-inflammatory activity of synthesized compounds may be due to the incorporation of triazole thiadiazole nucleus in to Ibuprofen moiety. The expected antibacterial activity was not observed where as it has resulted in to a good anti-inflammatiry activity. The excellent anti-inflammatory of synthesized compounds i.e. **$3a_3$,$3a_4$,$3a_8$** may be due to the presence of electron withdrawing group present in the 4^{th} position of coumarine nucleus, which is attached at 5^{th} of position thiadiazole ring.

CONCLUSION

A novel series of 1,2,4-triazolo [3,4-b] [1,3,4] thiadiazoles were prepared by treating 4-amino -3-substituted -5-mercapto-1,2,4-triazoles with 2-amino-4-(substituted phenyl)-3-carboethoxy benzopyron. Their chemical structures were confirmed by means of spectral data IR, ^{1}H NMR and Elemental analysis. All the synthesized compounds were screened for antimicrobial activity and anti-inflammatory activities. Some of the synthesized compounds have shown very good antimicrobial and anti-inflammatory activities. Further modification of substituents on the coumarine nucleus may result good antibacterial and anti-inflammatory agents.



Scheme 1: Synthesis of novel triazolothiadiazoles

Table 1: Physical Constants of the Synthesized Compounds, $3a_1$-$3a_{11}$

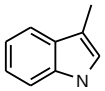
Compound	Subsistent (R)	Molecular formula	M.P.(^{0}C)	% of Yield
$3a_1$	C_6H_5	$C_{34}H_{30}N_5OS$	180	65
$3a_2$	2-Cl-C_6H_5	$C_{34}H_{29}ClN_5OS$	168	61
$3a_3$	4-Cl- C_6H_5	$C_{34}H_{29}ClN_5OS$	148	75
$3a_4$	4-OH- C_6H_5	$C_{34}H_{30}N_5O_2S$	240	56
$3a_5$	2-OMe- C_6H_5	$C_{35}H_{32}N_5O_2S$	156	75
$3a_6$	4-OMe- C_6H_5	$C_{35}H_{32}N_5O_2S$	170	75
$3a_7$	2-NO_2- C_6H_5	$C_{34}H_{30}N_5O_2S$	145	80
$3a_8$	4-NO_2- C_6H_5	$C_{35}H_{29}N_6O_3S$	160	67
$3a_9$		$C_{32}H_{28}N_5OS_2$	169	50
$3a_{10}$		$C_{33}H_{29}N_6OS$	179	69
$3a_{11}$		$C_{36}H_{31}N_6OS$	192	60

TABLE 2; Antibacterial activity of the Synthesized Compounds, $3a_1$-$3a_{11}$

Sample Code	*Inhibition zone diameter in mm							
	B.subtilis		***B.pumilis***		***E.coli***		***P.aureginosa***	
	50µg	**100µg**	**50µg**	**100µg**	**50µg**	**100µg**	**50µg**	**100µg**
$3a_1$	8	9	7	10	5	12	7	8
$3a_2$	9.5	14	7	12	6	14	6	10
$3a_3$	7	7	6	9	4	10	5	6
$3a_4$	6	10	8	8	9	9	10	12
$3a_5$	10	8.5	8	10	6	7	8	10
$3a_6$	6	9	8	5	10	6	11	12
$3a_7$	14	10	11	8	10	8	9	13
$3a_8$	7	6	10	6	9	9	8	13
$3a_9$	5	9	7	9	6	10	5	10
$3a_{10}$	3	8	6	12	5	11	4	10
$3a_{11}$	12	6	9	10	13	10	10	8
Ampicillin	14	24	13	23	14	22	14	23
DMF	-	-	-	-	-	-	-	-

Table 3: Data showing anti-inflammatory activity of Triazolo thiadiazole derivatives in Carrageenan induced acute rat paw oedema model

			Paw oedema volume							
			After 1st hr		After 2 nd hr		After 3rd hr		After 4th hr	
Group	Treatment	Dose Mg/kg	Mean	% ROV	Mean	% ROV	Mean	% ROV	Mean	% ROV
1	Control	0.4ml	0.76	-	0.85	-	0.98	-	1.07	-
2	Standard	20	0.45	40.78	0.43	49.41	0.39	60.20	0.32	70.09
3	$3a_1$	200	0.2	73.68	0.11	87.05	0.11	88.77	0.1	90.65
4	$3a_2$	200	0.15	80.26	0.13	83.88	0.10	89.79	0.08	92.52
5	$3a_3$	200	0.162	78.68	0.18	78.82	0.20	79.59	0.21	80.18
6	$3a_4$	200	0.112	85.26	0.11	86.82	0.10	89.79	0.06	94.20
7	$3a_5$	200	0.18	75.39	0.12	85.88	0.12	87.75	0.12	88.31
8	$3a_6$	200	0.13	81.97	0.13	83.88	0.10	89.79	0.1	90.65
9	$3a_7$	200	0.2	73.68	0.23	72.94	0.24	75.51	0.26	75.70
10	$3a_8$	200	0.22	70.39	0.2	76.47	0.18	81.63	0.15	85.98
11	$3a_9$	200	0.12	84.7	0.12	85.82	0.10	88.99	0.07	94.21
12	$3a_{10}$	200	0.17	75.59	0.12	86.78	0.12	86.75	0.12	89.32
13	$3a_{11}$	200	0.14	82.1	0.13	83.98	0.11	89.69	0.11	91.5

ROV- Reduction in paw oedema volume.

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