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SYNTHESIS, DETERMINATION OF PARTITION COEFFICIENT AND ANTIMICROBIAL ACTIVITY OF TRIAZOLO THIADIAZINYL BROMOCOUMARIN DERIVATIVES

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ABSTRACT

A series of triazolo-thiadiazinyl bromocoumarins (10a-k) were synthesized and the compounds were characterized by IR, ^{1}H-NMR and Mass spectral analysis. The partition coefficient, a hydrophobic parameter was also determined for the newly synthesized compounds and the same were subjected for their antimicrobial activity.

Keywords: Bromocoumarin, Triazole, Thiadiazine, Antimicrobial activity, Partition coefficient.

INTRODUCTION

Coumarin derivatives have recently revealed new biological activities with interesting potential in therapeutic applications, besides their traditional employment as anticoagulants[1] and sun tanning agents[2], they have yielded important results as antibiotics[3], anti- AIDS agents[4] and anti-tumor drugs[5] It was therefore, speculated to combine 6-bromo-3-(2-bromoacetyl)-2H-1-benzopyran-2-one (9) and substituted 1, 2, 4-Triazoles (8a-K). In the present investigation, antimicrobial activity and partition coefficient (Log p) were established for the synthesized compounds (10a-k).

EXPERIMENTAL

Melting points were determined in open capillary tubes and were uncorrected.IR spectra were recorded on Fourier Transform IR spectrophotometer (Shimadzu 8700)using KBr disc method. ^{1}H-NMR spetra were recorded on AMX-400 liquid state NMR spectrometer in $CDCl_3$ usingTMS as an internal standard. Mass spectra were recorded on LC-MS Triple Quadruple Mass Spectrometer (Sciex 3000, Applied Biosystems) using electro sprayionization-positive ion mode. The purity of the test compounds was determined by thin layer chromatography. The λ_{max} of the synthesized compounds was recorded on UV-Visible Spectrophotometer (SHIMADZU 1601, 200ηm to 400ηm). the compounds were analyzed for C, H, and N analysis and the values were found to be ± 0.4% of calculated values. Physical data of the compounds were recorded in Table-1. Qualitative antimicrobial studies were carried out by plate method and the results were recorded in Table-2.

4-Amino-3-mercapto-5-(substituted phenyl)-triazole (8a-k)

Various substituted aromatic acids (4a-k) were used for the synthesis of. 1,2,4-triazoles[6] (8a-k). the reation involved the conversion of acids to their corresponding methyl esters (5a-k), acid hydrazides (6a-k), potassium dithiocarbazate salts (7a-k) and finally to 1,2,4-triazoles (8a-k) (Scheme-1). The triazoles were recrystallized from aqueous ethanol(8a-l) and(8k) and from equeous DMF (8j). the formation of triazoles were confirmed by difference in m.p., R_f values and IR (KBr) at the range 3400-3200 cm^{-1} ν(N-H), 3100-3000 cm^{-1} ν(ArC-H),1633-1488 cm^{-1} ν (ArC=C),1300-1250cm^{-1} ν (C-N), 833, 739 cm^{-1} ν (ArC-H)

3-[5-substituted phenyl]-1,2,4-triazolo-[3,4]-thiadiazin-5-yl-6-bromo-2H-1-benzopyran-2-ones (10a-k)

6-bromo-3-(2-bromoacetyl)-2H-1-benzopyran-2-one (9) (0.01 mol) and substituted [1,2,4]-triazoles (8a-k) (0.01 mol) were refluxed with 10mL each of absolute ethanol and dimethyl

formamide for 2 hrs with catalytic amounts of pyridine. It was allowed to cool and the product (10a-k) obtained was filtered, washed with ethanol and recrystallizedfrom aqueous dimethyl-formamide. The formation of these substituted coumarins was confirmed by difference in m.p., R_f values and IR peaks.

The absence of bands for C=O str of $–COCH_2Br$ (9), NH_2 str (doublet) (8a-k) and S-H str (8a-k) in the final compounds (10a-k) indicated the completion of the reaction to yield the respective triazolo-thiadiazinyl coumarins. Compound (10a) showed IR (KBr) at 3035 cm^{-1} ν (ArC-H), 1722 cm^{-1} ν(lactone C=O), 1604, 1550 and 1465 cm^{-1} ν(ArC=C), 1242 cm^{-1} ν (C-N), 817, 773 cm^{-1} ν(ArC-H).

^{1}H-NMR of 10g: 8.23 (s, 1H, C-H of lactone),7.32-7.34 (d, 1H, Ar-H), 4.11 (s, 2H, S-CH_2).

Mass Spectra of 1-a: Peaks were observed at m/e 439 (M+), 441 (M+2) 443 (M+4) and at 229.4 (Base peak), Other peaks were observes at m/e: 415.1, 355, 312.8, 299.6, 271.4, 256.6, 9, 1, 163 and 125.0.

Antimicrobial Activity

Antibacterial acreening if the synthesized compounds (10a-k) was carried out by cup-plate method[7] using two species of Gram-positive bacteria namely, *Bacillus subtilis* and and Staphylococcus aureus and three species of Gram negative bacteria namely, *Escherichia coli, Klebsiella pneumonia and pseudomonas aeruginosa* as presented in Table-2 using amoxicillin as a standard drug.

Partition Coefficient

Hydrophobicity is generally parametrized by partition coefficient. It was determined by using the Classical Shake Method[8] using n-octanol and phosphate buffer (pH 7.4).

Partition coefficient is defined as the ratio of the drug present in organic phase to that present in the aqueous phase.

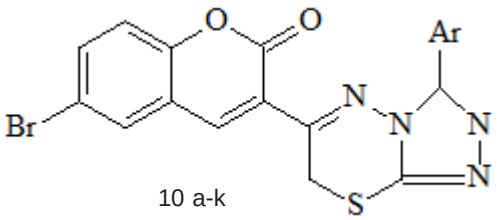
Partition coefficient = $P = C_{org}/ C/_{aq}$

$P=B_E/B_E-A_E$

Where, B_E= Absorbance before extraction

A_E= Absorbance after extraction

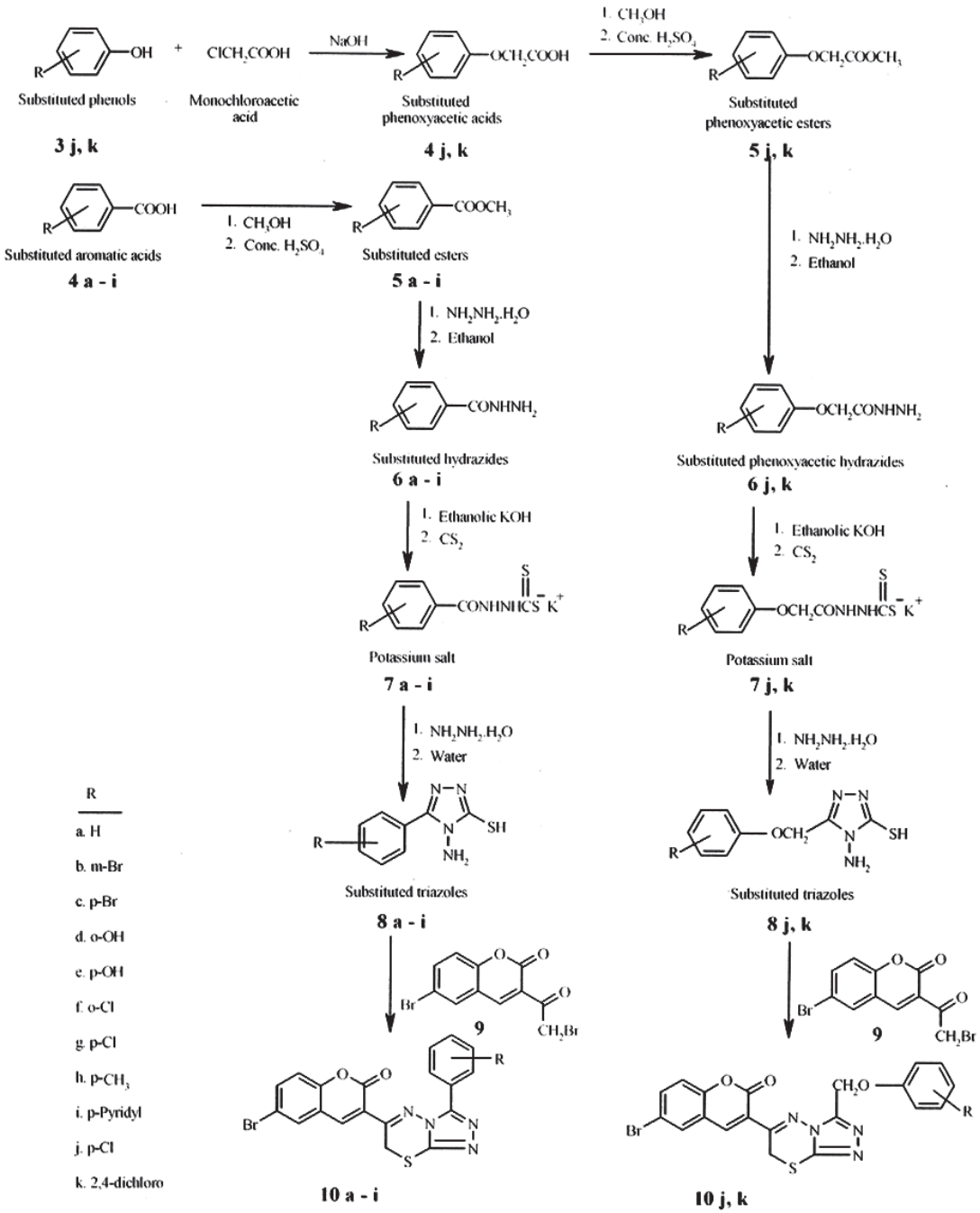
Table - 1 : Physical data of 3-(5-substituted phenyl)-1,2,4-triazolo-[3,4-b] [1,3,4]-thiadiazin-5-yl)-6-bromo-2H-1-benzopyran-2-ones (10a-k)



10 a-k

Comp.	Ar	Molecular Formula	MW	Mp	Yield	Log P
10a	Phenyl	$C_{19}H_{11}O_2N_4SBr$	439	252	65	1.412
10b	3-Bromophenyl	$C_{19}H_{10}O_2N_4SBr_2$	518	242	66	1.630
10c	4-Bromophenyl	$C_{19}H_{10}O_2N_4SBr_2$	518	246	69	1.580
10d	2-Hydroxyphenyl	$C_{19}H_{11}O_3N_4SBr$	455	>294	68	0.826
10e	4-Hydroxyphenyl	$C_{19}H_{11}O_3N_4SBr$	455	>294	72	0.645
10f	2-Chlorophenyl	$C_{19}H_{10}O_2N_4SClBr$	473.5	272	70	ND
10g	4-Chlorophenyl	$C_{19}H_{10}O_2N_4SClBr$	473.5	276	70	1.501
10h	4-Methylphenyl	$C_{20}H_{13}O_2N_4SBr$	453	242	66	1.806
10i	4-Pyridyl	$C_{18}H_{10}O_2N_5SBr$	440	250	65	1.220
10j	4-Chlorophenoxy	$C_{20}H_{12}O_3N_4SClBr$	503.5	222	67	1.421
10k	2, 4-Dichlorophenoxy	$C_{20}H_{11}O_3N_4SCl_2Br$	538	240	71	1.471

ND : Not Done


Substituted phenols
3 j, k
Monochloroacetic acid
Substituted phenoxyacetic acids
4 j, k
Substituted phenoxyacetic esters
5 j, k
Substituted aromatic acids
4 a - i
Substituted esters
5 a - i
Substituted hydrazides
6 a - i
Substituted phenoxyacetic hydrazides
6 j, k
Potassium salt
7 a - i
Potassium salt
7 j, k
Substituted triazoles
8 a - i
Substituted triazoles
8 j, k
9
10 a - i
10 j, k
R
a. H
b. m-Br
c. p-Br
d. o-OH
e. p-OH
f. o-Cl
g. p-Cl
h. p-CH3
i. p-Pyridyl
j. p-Cl
k. 2,4-dichloro

Scheme - 1

Log P was then calculated, which gives us an idea about the solubility and lipid profile of test sample.

RESULTS AND DISCUSSION

The antibacterial activity of all the triazole system. Further, the effect of smaller ring substitutions such as hydroxy, methyl, chloro and bromo showed varied activities. The effect of electron donating groups such as methyl and hydroxy substitutions on the aromatic ring did not significantly increase the antibacterial potency of the truiazolo-thiadiazinyl coumarins, however, the deactiveating halogen atoms showed marked increase in their antibacterial potency as depicted in Table - 2.

Test compounds showing promising antibacterial activity also have halogen substitution on the phenoxy ring attached to the triazole system. The zone of inhibition values for 2,4-dichlorophenoxy derivative (10k) were found to be 30mm, 34mm, 27mm, and 32 mm against *Bacillus subtilis*, *Staphyloccoccus aureus*, *Escherichia coli, Klebsiella pneumonea* and *Pseudomonas aeruginosa* respectively. However, pyridyl derivative (10i) also exhibited significant zone of inhibition values at 40mm, 40mm, 37mm, 37mm and 36mm against *Bacillius subtilis*, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* respectively using Amoxycillin as standard drug. The physical constants and coefficient values of the test compounds are tabulated in Table - 1.

Table - 2 : Antimicrobial activity of 3-(5-substituted phenyl)-1,2,4-triazolo-[3,4-b] [1,3,4]-6-bromo-2H-1-benzopyran-2-ones (10a-k)

Comp.	Zone of Inhibition (mm)				
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***
10a	10	10	8	8	5
10b	10	NA	8	8	NA
10c	14	15	14	10	9
10d	NA	NA	NA	NA	NA
10e	NA	NA	NA	NA	NA
10f	7	8	6	6	NA
10g	19	17	12	10	9
10h	7	10	NA	NA	NA
10i	40	40	37	37	36
10j	32	22	24	17	12
10k	30	34	27	27	32
AM	35	39	34	34	32

Standard: Amoxycillin: 100µg/0.1 mL; AM: Amoxycillin; NA: No Active

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