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Synthesis, Characterization and Determination of Partition Coefficient of Some Triazole Derivatives of Coumarins for Their Antimicrobial Activity

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The triazolo-thiadiazinyl coumarins have been synthesized by the action of 3-bromoacetyl coumarin and substituted triazoles. The synthesized compounds have been characterized by IR, ^{1}H-NMR and mass spectral analysis. An attempt to determine the hydrophobic parameter (partition coefficient) was also established. All the test compounds were screened for their antimicrobial activity by qualitative and quantitative method.

Key Words: Coumarin, Triazole, Thiadiazine, Antimicrobial activity, Partition coefficient.

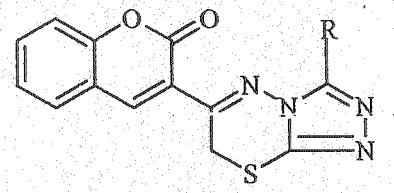
INTRODUCTION

The coumarin nucleus has been a seat of diverse biological properties through its innumerable derivatives. Compounds containing the benzopyran moiety have been associated with diverse pharmacological activities like antimicrobial, anti-tubercular, anticancer, analgesic and anti-inflammatory activities[1]. Triazole nucleus is also associated with various pharmacological activities like antimicrobial[2], antibacterial[3], antiviral and antifungal[4] effects. Coumarin derivatives with a heterocyclic system at position-3 are known to exhibit a significant change in their biological activity[5]. It is therefore speculated to link 3-substituted coumarins and 1,2,4-triazoles. Antimicrobial activity and partition coefficient (log P) were established for the synthesized test compounds.

EXPERIMENTAL

Melting points were determined in open capillary tubes and were found uncorrected. IR spectra were recorded on Fourier transform IR spectrophotometer (Shimadzu 8700) using KBr disc method. ^{1}H NMR spectra were recorded on AMX-400 liquid state NMR spectrometer in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on LC-MS "triple quadruple mass spectrometer (Sciex 3000, Applied Biosystems)" using electrospray ionization-positive ion mode. The purity of the test compounds was determined by thin layer chromatography. The λ_{max} of the synthesized compounds for determination of log P was recorded on UV-visible spectrophotometer (Shimadzu 1601, 200–400 nm). Quantitative antimicrobial studies were recorded on an Elisa microplate reader (Spectra Max 340 pc, molecular devices, 540 nm). The compounds were analyzed for C, H and N analysis and the values were found to be $\pm$ 0.4% of calculated values. Physical data of the compounds are recorded in Table-1.

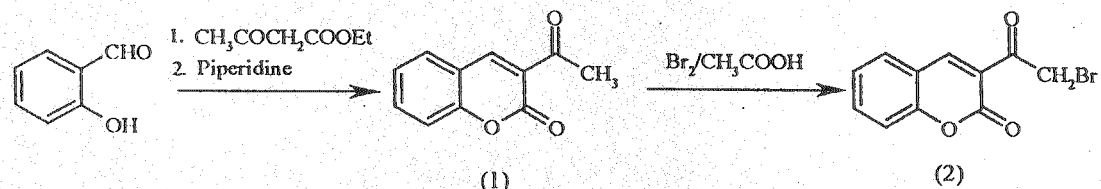
TABLE-1
PHYSICAL DATA OF 3-(5-(SUBSTITUTED PHENYL)-1,2,4-TRIAZOLO-[3,4-b][1,3,4]-THIADIAZIN-5-YL)-2H-1-BENZOPYRAN-2-ONES (**9a–l**)



(**9a–l**)

Comp.	R	m.f.	m.w. (g/m)	m.p. (°C)	Yield (%)	log P
9a	Phenyl	$C_{19}H_{12}N_4O_2S$	360	218	62	1.185
9b	Benzyl	$C_{20}H_{14}N_4O_2S$	374	196	65	1.088
9c	3-Bromophenyl	$C_{19}H_{11}N_4O_2SBr$	439	208	65	1.436
9d	4-Bromophenyl	$C_{19}H_{11}N_4O_2SBr$	439	274	67	–
9e	2-Hydroxyphenyl	$C_{19}H_{12}N_4O_3S$	376	> 294	68	0.596
9f	4-Hydroxyphenyl	$C_{19}H_{12}N_4O_3S$	376	> 294	66	0.381
9g	2-Chlorophenyl	$C_{19}H_{11}N_4O_2SCl$	394.5	240	70	1.359
9h	4-Chlorophenyl	$C_{19}H_{11}N_4O_2SCl$	394.5	276	70	1.421
9i	4-Methylphenyl	$C_{20}H_{14}N_4O_2S$	374	230	66	1.656
9j	4-Pyridyl	$C_{18}H_{11}N_5O_2S$	361	230	65	1.048
9k	4-Chlorophenoxy	$C_{20}H_{13}N_4O_3SCl$	424.5	196	62	1.307
9l	2,4-Dichlorophenoxy	$C_{20}H_{12}N_4O_3SCl_2$	459	212	71	1.340

3-Acetylcoumarin (1): A mixture of salicylaldehyde (63 g, 0.5 mol) and ethylacetoacetate (61 g, 0.5 mol) was taken in a conical flask, stirred and cooled. To this mixture, 10 g of piperidine was added with shaking. The mixture was then maintained at freezing temperature for 2–3 h and the yellow coloured solid mass separated out were broken in cold ethanol and filtered. The solid was washed with cold ethanol and dried to give 3-acetylcoumarin (55 g, 84%) (**Scheme-1**). The product was recrystallized from hot glacial acetic acid which yielded needle-shaped crystals (m.p. 118°C). The formation of this compound was confirmed by difference in m.p., R_f values and IR peaks (cm^{-1}) at 3034 ν(ArC=C), 1738 ν(lactone, C=O), 1678 ν(C=O of —$COCH_3$), 1678, 1556, 1492 ν(ArC=C), 1211 ν(C—O), 857, 758 ν(ArC—H).



(1) (2)

Scheme-1

3-Bromoacetyl coumarin (2): To a solution of compound **1** (18.8 g, 0.1 mol) in 150 mL of glacial acetic acid, bromine (16 g, 0.1 mol) in 20 mL of glacial acetic acid was added with stirring for 30 min at room temperature. The mixture was then

warmed to decompose an addition product. The mixture was heated for 15 min on a water-bath to expel most of the hydrogen bromide, cooled and filtered. It was then poured into ice-cold water and the solid (20.2 g, 76%) separated out was filtered, washed with water and dried. The product was washed with ether and recrystallized from chloroform. The formation of this compound was confirmed by difference in m.p., R_f values and IR peaks (cm^{-1}) at 3038 ν(ArC═C), 1728 ν(lactone, C═O), 1693 ν(C═O of —$COCH_2$), 1627, 1552, 1493 ν(ArC═C), 1238 ν(C—O), 873, 759 ν(ArC—H), 548 ν(ArC—Br).

4-Chlorophenoxy acetic acid (4k) and 2,4-dichlorophenoxy acetic acid (4l)[6]: To a mixture of **3k** (6.4 g, 0.05 mol) and monochloroacetic acid (4.7 g, 0.05 mol), a solution of sodium hydroxide (4.5 g, 0.112 mol) in 25 mL of water was added slowly with continuous stirring. Considerable heat was generated during the reaction. The reaction mixture was heated on a wire-gauze until most of the liquid evaporated. 150 mL of water was added to the residue. The solution was cooled and filtered. The clear solution was acidified to Congo red with concentrated hydrochloric acid. The separated dense oil was extracted with 2 × 25 mL portions of ether. The combined extract was washed with 10–15 mL of water and dried over anhydrous magnesium sulfate. Magnesium sulfate was removed by filtration and ether was distilled off to give 7 g of 4-chlorophenoxy acetic acid (**4k**) (76%). They were recrystallized from boiling water. Similar procedure was used to synthesize the other acid (**4l**) and recrystallization was done by boiling water. The formation of these compounds was confirmed by difference in m.p., R_f values and IR peaks (cm^{-1}) at 3310 ν(O—H), 3040 ν(ArC═C), 1728 ν(C═O), 1627, 1552, 1493 ν(ArC═C), 1238 ν(C—O), 873, 759 ν(ArC—H), 744 ν(C—Cl).

4-Amino-3-mercapto-5-(substituted phenyl)-triazole(8a–l): The various substituted aromatic acids (**4a–l**) were used for the synthesis of 1,2,4-triazoles[7] (**8a–l**). The reaction involved conversion of acids to their methyl esters (**5a–l**), acid hydrazides (**6a–l**), potassium dithiocarbazate salts (**7a–l**) and finally to 1,2,4-triazoles (**8a–l**). The triazoles were recrystallized from aqueous ethanol (**8a–i, k, l**) or from aqueous DMF (**8j**). The formation of these triazoles was confirmed by difference in m.p., R_f values and IR peaks (KBr, cm^{-1}) at 3249, 3147 ν(N—H), 3028 ν(ArC═C), 1633, 1596, 1488 ν(ArC═C), 1311 ν(C—N), 833, 739 ν(ArC—H).

3-(5-(Substituted phenyl)-1,2,4-triazolo-[3,4-b] [1,3,4]-thiadiazin-5-yl)-2H-1-benzopyran-2-ones (9a–l): Compound **2** (2.6 g, 0.01 mol) and [1,2,4]-triazoles (0.01 mol) (**8a–l**) were refluxed with 10 mL each of absolute ethanol and dimethyl formamide for 2 h with catalytic amounts of pyridine. It was allowed to cool and the product (**9a–l**) obtained was filtered, washed with ethanol and recrystallized from 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 ν(C═O) of —$COCH_2$ (**2**), ν(N—H) (doublet) (**8a–l**) and ν(S—H) (**8a–l**) in the final compounds (**9a–l**) indicated the completion of the reaction to yield the respective triazolo-thiadiazinyl coumarins. Compound (**9a**) showed IR peaks (KBr, cm^{-1}) at 3044 ν(ArC═C), 1726 ν(lactone C═O), 1606, 1558, 1461 ν(ArC═C), 1371 ν(C—N), 833, 765 ν(ArC—H).

^{1}H NMR: 9a: 8.28 (s, 1H, C—H of lactone), 7.40–7.64 (m, 4H, Ar—H), 7.20–7.32 (m, 5H, Ar—H), 4.12 (s, 2H, S—CH_2).

9b: 8.14 (s, 1H, C—H of lactone), 7.38–7.70 (m, 4H, Ar—H), 7.25–7.32 (m, 5H, Ar—H), 4.32 (s, 2H, —CH_2—Ph), 4.03 (s, 2H, S—CH_2).

9d: 8.32 (s, 1H, C—H of lactone), 7.93–7.96 (d, 2H, Ar—H, J = 8.61 Hz), 7.37–7.71 (m, 4H, Ar—H + m, 2H, Ar—H, J = 8.599 Hz), 4.12 (s, 2H, S—CH_2).

9f: 8.24 (s, 1H, C—H of lactone), 7.93–7.97 (d, 2H, Ar—H, J = 8.634 Hz), 7.35–7.76 (m, 4H, Ar—H + m, 2H, Ar—H, J = 8.637 Hz), 4.12 (s, 2H, S—CH_2).

9h: 8.32 (s, 1H, C—H of lactone), 8.01–8.03 (d, 2H, Ar—H, J = 8.714 Hz), 7.37–7.71 (m, 4H, Ar—H + d, 2H, Ar—H, J = 7.696 Hz), 4.12 (s, 2H, S—CH_2).

9k: 8.30 (s, 1H, C—H of lactone), 7.88–7.93 (d, 2H, Ar—H, J = 8.630 Hz), 7.30–7.76 (m, 4H, Ar—H + d, 2H, Ar—H, J = 7.633 Hz), 5.12 (s, 2H, CH of —OCH_2—Ph), 4.12 (s, 2H, S—CH_2).

Mass Spectra: 9b: Peaks were observed at m/e 375.1 (M + l) and at 63.8 (Base peak).

Antimicrobial activity: Antibacterial screening of the synthesized compounds was carried out by cup-plate method[8] using 2 species of gram-positive bacteria (*B. subtilis* and *S. aureus*) and three species of gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa*). Antibacterial activity of compounds (**9a–l**) is presented in Table-2. The MIC of compounds showing good antimicrobial activity was determined using 96-well plate (two-fold dilution technique) using an Elisa Reader[9].

TABLE-2
ANTIMICROBIAL ACTIVITY OF 3-(5-(SUBSTITUTED PHENYL)-1,2,4-TRIAZOLO-[3,4-b] [1,3,4]-THIADIAZIN-5-YL)-2H-1-BENZOPYRAN-2-ONES (**9a–l**)

Compd.	Zone of inhibition (in mm)					Inhibition compared (%)									
						Amoxicillin					Gentamycin				
	B.s.	*S.a.*	*E.c.*	*K.p.*	*P.a.*	*B.s.*	*S.a.*	*E.c.*	*K.p.*	*P.a.*	*B.s.*	*S.a.*	*E.c.*	*K.p.*	*P.a.*
9a	9	9	7	7	4	–	–	–	–	–	–	–	–	–	–
9b	38	32	32	35	31	89	52	53	71	43	82	47	48	60	36
9c	12	–	12	–	–	–	–	–	–	–	–	–	–	–	–
9d	19	17	17	12	11	–	–	–	–	–	–	–	–	–	–
9e	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9f	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9g	6	8	4	5	4	–	–	–	–	–	–	–	–	–	–
9h	16	14	16	10	10	–	–	–	–	–	–	–	–	–	–
9i	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9j	42	43	40	42	42	132	152	104	166	144	114	136	98	140	120
9k	26	22	18	19	16	–	–	–	–	–	–	–	–	–	–
9l	22	36	29	18	34	29	145	53	39	150	26	130	48	32	125
Am	38	36	36	35	40	–	–	–	–	–	–	–	–	–	–
Ge	37	37	38	39	41	–	–	–	–	–	–	–	–	–	–

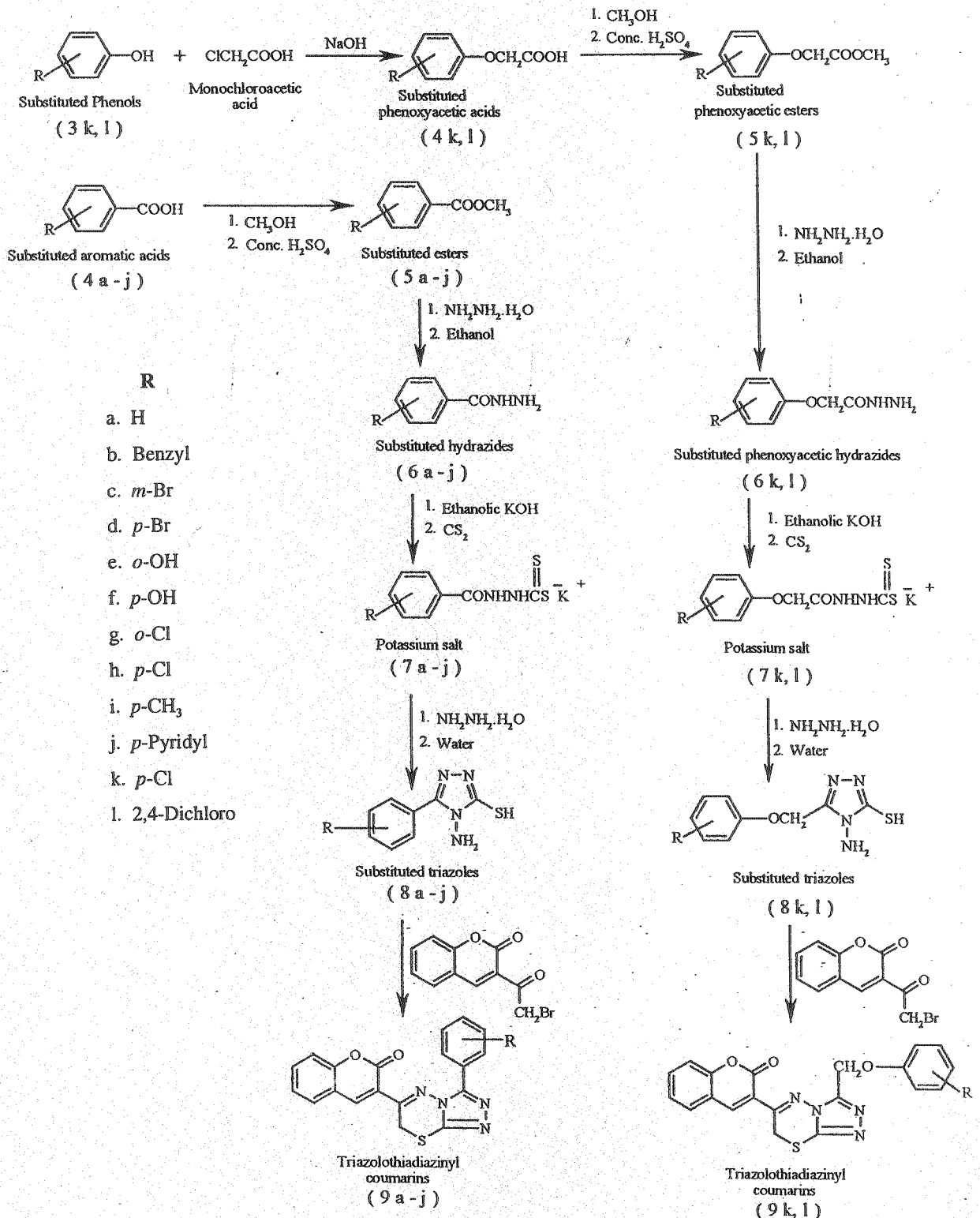
B.s.: *B.subtilis*, *E.c.*: *E.coli*, *K.p.*: *K. pneumoniae*, *P.a.*: *P. aeruginosa* *S.a.*: *S. aureus*,
Am: Amoxycillin, Ge: Gentamycin

Partition coefficient: Hydrophobicity is generally parametrized by partition coefficient or some derivative of partition coefficient. It was determined by using the classical shake method[10] using *n*-heptanol and phosphate buffer (pH 7.4).

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

$$\text{Partition coefficient} = P = C_{org}/C_{aq} = B_E/(B_E - A_E)$$

where, B_E = absorbance before extraction, A_E = absorbance after extraction.



Scheme-2

RESULTS AND DISCUSSION

All the synthesized compounds were evaluated for their antibacterial activity by cup-plate method. Test compounds such as **9c**, **9e**, **9f** and **9i** did not show any notable activity. Test compounds such as **9a**, **9d**, **9k**, **9h** and **9j** showed activity less than the standard antibiotic. However, test compounds such as **9b**, **9j** and **9l** showed activity comparable with that of standard antibiotics against all the species. Quantitative evaluation of antimicrobial activity of these compounds was carried out using two-fold dilution technique and the antibacterial activity of **9j** was found to be very promising.

Test compounds such as **9e** and **9f** possessed least partition coefficient due to the presence of hydroxy group on the phenyl ring attached at 5th position of triazole ring. However, compound **9i** carrying an electronegative atom on the phenyl ring showed the maximum log P value. Compounds **9c**, **9d**, **9g** and **9h** possessed value nearing to that of **9i**. Increase in the electronegativity of the substituents on the aromatic ring showed increase in the lipophilicity of the test compounds (Br > Cl). Further, the substituents with an electronegative atom at *p*-position (**9d** and **9h**) showed an increased log P values than that of *o*- or *m*-substitutions (**9c** and **9g**). log P of the other compounds were well within the range of 1 and 1.3. Compound **9l** showed an increase in log P value over that of **9k**. This can be explained due to an attachment of electronegative substituents on the phenoxy ring.

Thus it was observed that the test compounds of triazolo-thiadiazinyl coumarins with partition coefficient values in the range of 1–2 might have antibacterial activity better than the test compounds possessing very high partition values.

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