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Synthesis and Studies on Biologically Active Mono and Biheterocycles

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A new series of 1-(N-heteroyl/diphenyl amino acetyl/propionyl) benzotriazoles (3a-h) and 4-(N-heteroyl/diphenyl/amino acetyl/propionyl) morpholines (4a-h) have been synthesized by appropriate methods. All the newly synthesized compounds were characterized by their microanalysis, IR, ^{1}H-NMR and mass spectra and were also screend for their anthelmintic and antimicrobial activities.

THE heterocyclic compounds are important in chemistry and in pharmaceutical industry. The recent literature is enriched with progressive finding on the synthesis and pharmacological action of fused heterocycles.[1] The benzimidazole, benzotriazole, phenothiazine, morpholine and diphenylamine nucleus are associated with diverse pharmacological activities such as anti- inflammatory, antihistaminic, anticonvulsant, antineoplastic, anthelmintic and antimicrobial activities. Several compounds have been synthesized containing the biheterocycles moieties. The biological importance of benzotriazole, morpholine and its derivatives have encouraged the authors to synthesize several new additional compounds possessing these moieties.[2,3] the sequence of reactions leading to the synthesis of compounds (3a-h) and (4a-h) are shown in **scheme**. The starting materials were benzimidazole, benzotriazole, phenothiazine, and diphenylamine treated separately with α-chloroacetic/propionic acid, respectively to get the corresponding acids (1a-h). These acids were then converted into their chlorides (2a-h) by using thionyl chloride. The respective chlorides were allowed to react either benzotriazole or morpholine in alkaline medium to obtain the titled compounds (3a-h) and (4a-h). The structure of these compounds has been supported by their microanalyses. IR and in some cases by ^{1}H-NmR and mass spectral data. The characterization data are given in **Table-I**. All the synthesized compounds were screened for their anthelmintic and antimicrobial activities.

Experimental

Melting points were determined on a Toshniwal melting point apparatus and are uncorrected. Purity of compounds was checked by TLC on silica gel 'G' plates. IR spectra (KBr, cm^{-1}) were recorded on an Acculab-10-spectrophotometer. ^{1}H-NMR spectra in $CDCl_3$ on a CFT-20 instrument using TMS as internal standard (chemical shifts in δ, ppm) and mass spectra on a Jeol D-300 spectrometer.

Synthesis of the N-(benzimidazole-1-v-1) acetic acid (Ia)[4,5] General Procedure

The equimolar quantity of benzimidazole (5.95g, 0.05 mole) dissolved in 20-30 ml of 4N NaOH solution and α- chloroacetic acid were refluxed on a water bath for about 5-6 hours. The reaction mixture was then transferred into a beaker and was evaporated almost to dryness. The residue was then dissolved in 100 ml. water. The solution was acidified with dilute hydrochloric acid to yield the free acid. The product was recrystallized from benzene to furnish (Ia), yield (64%), m.p. 110-112°; Found; C, 61.34; H,4.42; N,15.78; $C_9H_8N_2O_2$; Calcd;C,61.36; H, 4.50;

Table-I: Physical and anlytical data of the compounds (1b-h), (2b-h), (3a-h) and (4a-h).

Compds. No.	Yield (%)	M.P. (°C)	^{1}H-NMR δ,ppm (90 MHz)
1b	58	90-92	—
1c	66	118-120	—
1d	62	118-120	—
1e	65	130-132	—
1f	60	120-122	—
1g	68	80-82	—
1h	64	68-70	—
2b	76	124-126	—
2c	71	136-138	
2d	63	108-110	—
2e	80	140-142	—
2f	74	126-128	—
2g	85	156-158	—
2h	82	138-140	—
3a	64	114-116	3.80(s, 2H, CH_2); 7.20-8.15(m, 9H,Ar-H)
3b	58	104-106	—
3c	62	110-112	—
3d	55	98-100	—
3e	67	124-126	—
3h	65	108-110	—
3g	71	88-90	—
3h	66	74-76	3.30(q, 1H, CH); 1.80(d, 3H, CH_3); 6.80-7.60(m.14H,Ar-H).
4a	58	90-92	4.05(s, 2H, CH_2); 2.82(s, 4H, 2xCH_2): 3.40(s, 4H 2xCH_2); 7.10-7.90(m,5H,Ar-H).
4b	52	78-90	—

4c	54	96-98	—
4d	50	79-81	—
4e	60	102-104	3.90(s, 2H, CH_2); 2.88(s, 4H, 2xCH_2; 3.55(s, 4H, 2xCH2); 6.90-7.80(m,8H,Ar-H).
4f	57	94-96	—
4g	63	75-77	—
4h	59	64-66	—

All new compounds showed satisfactory elemental analyses and spectra properties.

KBr
N, 15.90%. ν_{max} cm^{-1}, 1720 (–C–of COOH); 1450, 2880 (- cH_2); 1560 (C=C); 1620 (C=N); 3140 (C-H of aromatic). Similarly other acetic/propionic acid (1b-h) were prepared and characterized. (Table-I).

Synthesis of the N-(benzimidazol-1-yl) acetyl chloride (1b)[6,7] General Procedure

A solution of N-(benzimidazole-1-1-yl) acetic acid (4.52g, 0.038 mole) in benzene (20 ml) and thionyl chloride (0.075 mole) was refluxed for about 8 hours on a waterbath. Excess of thionyl chloride was distilled off under reduced pressure, cooled and separated chloride (2b) was recrystallized from $CHCl_3$, yield (85%), m.p. 136-138°; found; C, 55.49; H, 3.58; N, 14.35; $C_9H_7N_2OCl$; Calcd; C, 53.53; H, 3.60; N, 14.40% KBr ν_{max} : 1790 (–C–Cl); 1460, 2875 (-CH_2); 1565 (C=C); 1635 (C=N); 3120 (C-H of aromatic).

In the same manner, other chlorides (2b-h) were prepared and characterized treating (1b-h) with thionyl chloride (Table-I).

Synthesis of the N-(benzimidazol-1-yl) acetyl benzotriazole (3a)[8]

To an ice-cold solution of benzotriazole (2.7 g, 0.023 mole) and 5 ml of 4N sodium hydroxide solution in aqueous acetone was added dropwise in N-(benzimidazole-1-1-yl) acetyl chloride and contents were stirred on a magnetic strirrer at room temperature for about 4-5 hours. It was then kept overnight. The precipitate was filtered, washed with water and passed through a column of silica gel and eluted with pet-ether: benzene (6:4v/v). Finally it was recrystallized from ether. The compounds (3b-h) and (4a-h) were prepared in a likewise fashion using benzotriazole and morpholine (**Table-1**)

Pharmacological Evaluation

Anthelmintic Activity: The compounds (3a-h), (4a-h) were screened for their anthelmintic activity against the experimental infection of **Ancylostoma ceylanicum** in hamsters and **Hymenolepis nana** in young male rats (weighing 30-40g) respectively by Steward method.[9] Mebendazole was used as a standard drug which killed 100% of **A. ceylamicum** at an oral dose of 250 mg/kgx3 and 100% worms were cleared up in **H. nana** infection at an oral dose of 250 Mg/Kg. The percentage efficacy was calculated by the following formula.

$$\text{Percentage efficacy} = \frac{N-n}{N} \times 100$$

Where : N = Average number of worms recorded in control group.

n = Average number of worms recorded in treated group.

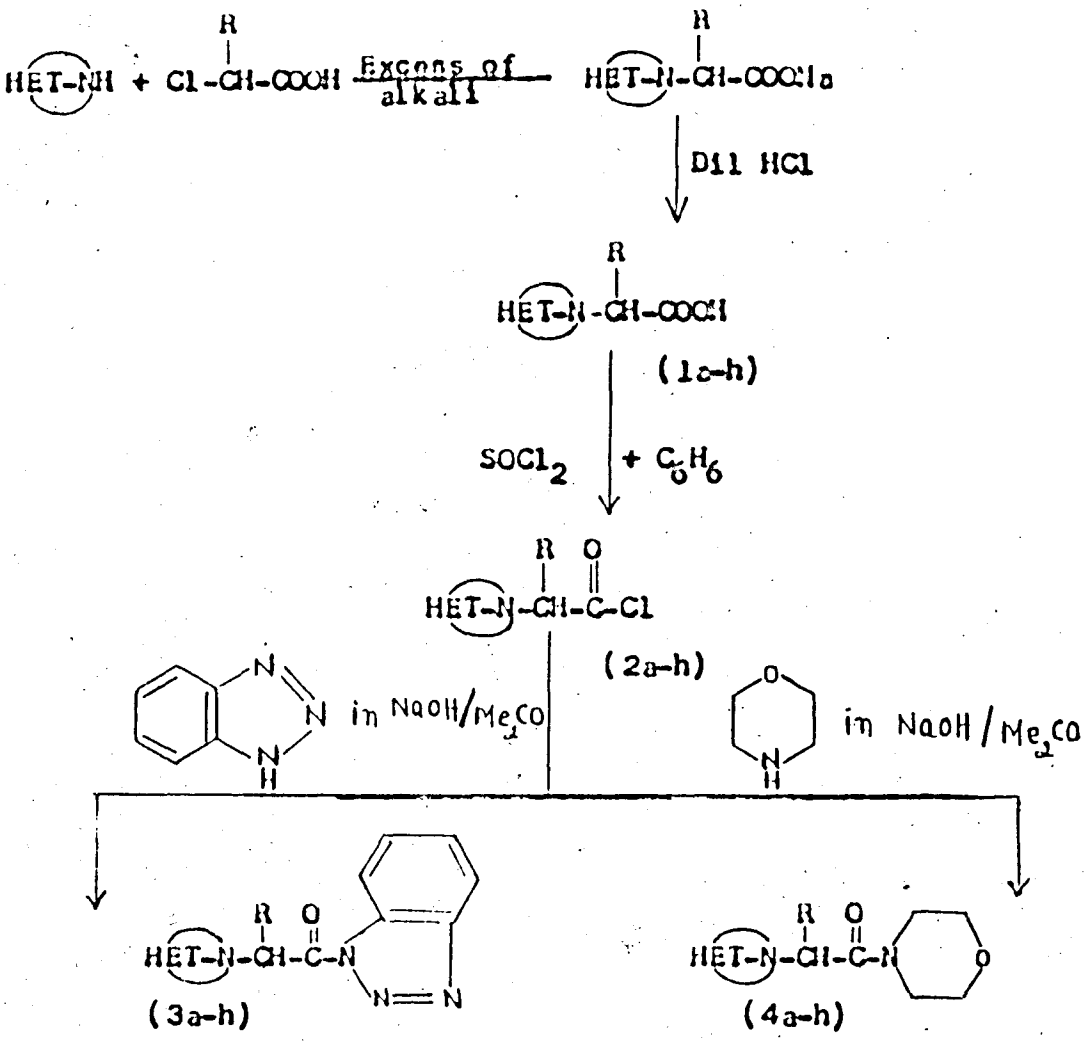

Chart : 1, 2, 3 and 4

	HET-NH	R
a	Benzimidazole	H
b	Benzimidazole	CH_3
c	Benzotriazole	H
d	Benzotriazole	CH_3
e	Phenothiazine	H
f	Phenothiazine	CH_3
g	Diphenylamine	H
h	Diphenylamine	CH_3

Table-II: Anthelmintic evaluation data of the compounds (3a-h) and (4a-h).

Compounds number	Ancyclostoma ceylanicum		Hymenolepis nana	
	Dose mg/kg	% worm reduction	Dose mg/kg	Active/Inactive
3a	250x3	72	250x1	Active
3b	250x3	76	250x1	Active
3c	250x3	42	250x1	Inactive
3d	250x3	53	250x1	Active
3e	250x3	78	150x1	Active
3f	250x3	83	150x1	Active
3e	250x3	22	250x1	Inactive
3h	250x3	16	250x1	Inactive
4a	250x3	52	250x1	Active
4b	250x3	56	250x1	Active
4c	250x3	32	250x1	Inactive
4d	250x3	28	250x1	Inactive
4e	250x3	65	250x1	Active
4f	250x3	68	250x1	Active
4g	250x3	15	250x1	Inactive
4h	250x3	21	250x1	Inactive

Mebendazole cleared up 100% worms in A. ceylamicum as well as H. nana.

The compounds derived from benzimidazole and phenothiazine nucleus 3a, 3b, 3e, 3f, 4a, 4b, 4e, and 4f eliminated 70-85% worms at a dose of 250 mg/kgx3 against **A. ceylanicum** and a dose of 250 mg/kg against **H. nana**. While other compounds displayed moderate anthelmintic activity. The results are recorded in **Table-II**.

Antimicrobial Activity: The compounds (3a-h), (4a-h) were also screened for their antibacterial activity against **Escherichia coli, Staphylococcus aureus** and **Bacillus anthracis**. The filter paper disc method[10] was employed using ethanolic solution of the compounds at 25 ug/ml and 50 ug/ml concentrations. Streptomycin was also used as a reference drug. For antifungal activity screened against **Asperquillus fumigatus, Candida albican and micrsporum gypseum** by the standard method[11] using mycostatin as a standard drug. In general compounds containing benzotriazole or morpholine nucleus are found to be significant and quite comparable with a standard drug. The results of these tests are expressed in **Table - III**.

Structure activity relationships : A large number of structural variation carried out in the hetero-

Table-III: Antimicrobial evaluation data of the compounds (3a-h) and (4a-h)

Comp. No.	Antibacterial activity						Antifungal activity					
	E. coli		S. Aureus		B. Anthracis		A. fumigatus		C. Albican		M. Gyspeum	
	25 µg/ml	50 µg/ml	25 µg/ml	50 µg/ml	25 µg/ml	50 µg/ml	25 µg/ml	50 µg/ml	25 µg/ml	50 µg/ml	25 µg/ml	50 µg/ml
3a	+	++	++	+++	+	++	+	++	-	+	+	++
3b	++	+++	++	+++	+	++	+	++	-	-	-	-
3c	++	+++	+++	++++	++	+++	++	+++	+	++	+	++
3d	+++	++++	++	+++	++	+++	++	+++	+	++	++	+++
3e	+	++	++	+++	+	++	-	+	+	++	-	-
3f	++	+++	+	++	++	+++	-	-	-	+	+	++
3g	++	+++	++	+++	+++	++++	+	++	+	++	++	+++
3h	+	++	++	+++	+	++	++	+++	++	+++	+	++
4a	++	+++	+	++	+	++	++	+++	+	++	+	++
4b	+	++	++	+++	+	++	++	+++	++	+++	+	++
4c	++	+++	+++	++++	++	+++	+++	++++	+	++	++	+++
4d	++	+++	+++	++++	+	++	+++	++++	+	++	++	+++
4e	+	-	+	++	-	-	++	+++	+++	++++	++	+++
4f	-	-	+	++	++	++	+	++	-	-	-	-
4g	+	-	+	++	-	-	-	+	+	++	+	++
4h	+	++	-	-	-	+	+	++	-	+	+	++
Stereto-mycin	++	+++	+++	++++	+++	++++	++	+++	+	++	++	+++
Myco-statin							+++	++++	++	+++	+++	++++

Zone of inhibition : + = 3/9 mm; ++ = 10-12 mm; +++ = 13-16 mm; ++++ = 17-21 mm. –= Not meausrable activity.
Streptomycin: 17 mm (25 ug/ml); 21 mm (50 ug/ml) and Mycostatin : 15 mm(25 ug/ml); 20 mm (50 ug/ml).

cyclic series have resulted in the establishment of some fairly consistent patterns of relationships between structure and activity. The nature and position of substituent of heterocyclic nucleus strongly influence activity. Heterocycles bearing functional group (NH) are important synthons in organic synthesis as well as medicinal chemistry. The presence of amino group on biologically active heterocycles would be quite rewarding from synthetic and biological aspects. So many compounds are synthesized which have the biheterocycle moiety substitution and found to be antiparasitic and antimicrobial activities. However, relatively little work has been done on heterocycles with an acetyl group directly attached to the heterocyclic ring, particularly to the ring nitrogen.[12]

The SAR show significant effects caused by modification of the length of both aliphatic chains. The two carbon side chain connecting the nitrogen of

heterocycle ring is more basic side chain. branching at the ∝-position of side chain with a small group such as methyl substitution ehhances the biological activity.

Conclusion: The procedure adopted in the synthesis of title compounds has a wide scope and allows straightforward synthesis of various biologically significant benzotriazole and morpholine derivatives carrying acetyl and propionyl functional groups.

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