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Synthesis and antibacterial activity of some new azopyrazoles

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Mannich Reaction of benzotriazole (1), ethyl-4-amino benzoate (2) and formaldehyde in ethanol afford 4-(1H)-benzotriazoyl methyl amino benzoate (3),which on treatment with hydrazine hydrate in the presence of ethanol results in 4-(1H)-benzotriazoyl methyl amino benzoyl hydrazide (4). This compound was produced by condensation with pre-prepared various ethyl-2-substituted phenyl hydrazono-3-oxobutyrate (6a-h), and yielded 1-(4-((1H)-benzotriazolyl methyl amino benzoyl)-3-methyl-4-(2-substituted phenyl hydrazono)-1H-pyrazoline-5(4H)-one (7a-h). All the compounds (7a-h) were characterized by IR and NMR spectral studies. The compounds showed significant antimicrobial activity against various bacteria and fungi.

Key words: 5-pyrazolinone, benzotriazole, antimicrobial activity, Mannich reaction, and spectral study.

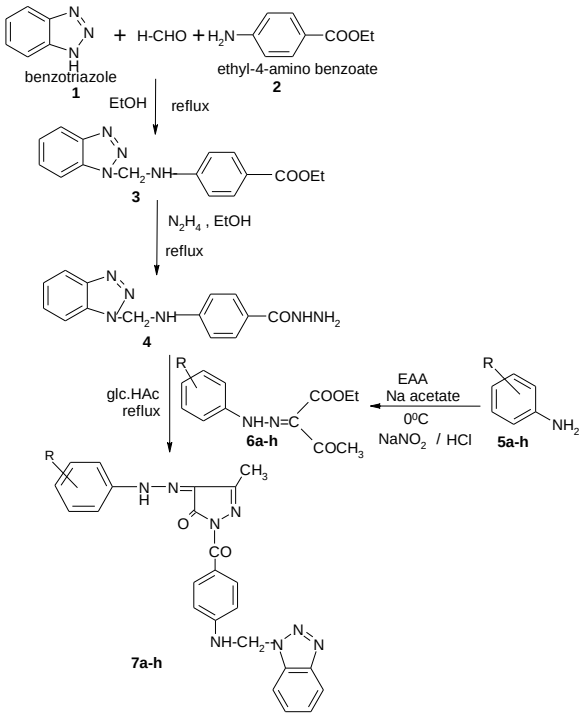
INTRODUCTION

The arylazopyrazoles are generally prepared by combination of aryl-azo-ethyl actoacetate derivatives and hydrazine derivatives [1-6]. Another heterocyclic compound, say benzotriazole, is found to be important. Its prime application is in the composition of the corrosion inhibitors for copper or copper alloys [7, 8]. Ciba Geigy has introduced benzotriazole derivative under the trade name of Trinvin-P [9]. It is applied as an UV light absorber for stabilizing plastics and other organic materials against discoloration determination. It is employed as photographic emulsion stabilizer [10]. In the peptide synthesis it acts in the form of an active ester [11]. The area of application where the merged molecule likes arylazopyrazole-benzotriazole has not been developed despite of their good biological properties. Hence this paper examines the synthesis and characterization of arylazopyrazole-benzotriazole derivatives, shown in Scheme1.

EXPERIMENTAL

Materials

All chemicals used were of laboratory grade. Benzocain (i.e.ethyl-4-amino benzoate) and Benzotriazole were prepared by the reported method. [6]



Scheme 1

R= (a) 4-H; (b) 2-CH_3; (c) 4-Cl; (d) 4-Br; (e) 4-Nitro; (f)2,4-Dinitro; (g)2,4-Dichloro-6-Nitro; (h)2,4,6-tribromo.

Measurement

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded in KBr pellets on a Nicolet 760D spectrometer, and ^{1}H NMR and ^{13}C NMR spectra

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Table 1. Physical and analytical data of the synthesized (6a-h) compounds.

Compound No.	R	Molecular Formula	M.P. °C	Yield %	Elemental Analysis						Additional ^{1}HNMR Signals (δ , ppm)
					C%		H%		N%		
					Found	Calcd.	Found	Calcd.	Found	Calcd.	
6a	H	$C_{11}H_{13}N_3O_3$	112	76	63.13	63.15	6.12	6.14	17.79	17.87	6.46-7.02 (s,5H,ArH)
6b	2-Me	$C_{12}H_{15}N_3O_3$	110	63	64.44	64.46	6.60	6.61	16.59	16.86	2.36 (s,3H,CH_3) 6.34-6.83 (s,4H,ArH)
6c	4-Cl	$C_{11}H_{12}N_3O_3Cl$	124	74	53.61	53.63	4.81	4.84	18.79	19.00	6.4-7.03 (s,4H,ArH)
6d	4-Br	$C_{11}H_{12}N_3O_3Br$	114	82	45.97	46.00	4.13	4.15	13.25	13.37	6.35-7.20 (s,4H,ArH)
6e	4-NO_2	$C_{11}H_{12}N_4O_5$	118	66	51.59	51.61	4.63	4.65	19.97	20.00	6.71-7.94 (s,4H,ArH)
6f	2,4-Dinitro	$C_{11}H_{14}N_5O_7$	124	59	44.42	44.44	3.67	3.70	21.45	21.73	6.98-8.38 (s,3H,ArH)
6g	2,4-Dichloro-6- Nitro	$C_{11}H_{10}N_4O_5Cl_2$	126	77	41.35	41.37	3.14	3.16	16.01	16.09	7.42-7.36 (s,2H,ArH)
6h	2,4,6-Tribromo	$C_{11}H_{10}N_3O_3Br_3$	122	84	30.54	30.57	2.31	2.33	8.78	8.89	7.30 (s,2H,ArH)

were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz and 100 MHz, respectively. LC-MS of selected samples were taken on LC-MSD-Trap-SL_01046. The compound purity was checked by TLC on silica gel plates, and the spots were visualized by exposure to iodine vapours.

Synthesis of 4 - (1H) - benzotriazolyl methyl amino benzoate (3)

A mixture of 1H- Benzotriazole (1) (0.02mole), formaldehyde (0.02mole) and benzocain (2) (0.02mole) in ethanol (50ml) was heated under reflux for 4 hrs. Subsequently, the ethanol was distilled off and the obtained pasty mass obtained was triturated with petroleum ether (40-60˚C). The solid compound 3 (see Scheme 1) was isolated, and dried. The yield was 62%, M.P.146–47ºC (uncorrected). (Found 64.60 %, H 5.25 %, N 18.75 %; Calcd. C 64.85 %, H 5.44 %, N 18.91 %).

IR spectra: 3030-3088 cm^{-1} (C-H of Ar.),1620-1489 cm^{-1} (C-C of Ar.) 2960,1380 cm^{-1} (-CH_3,-CH_2),1705–1765(C=O), 3450–3550 cm^{-1}(-NH-), 1375–1350 cm^{-1} (-CN),1196-1154 cm^{-1} (C-O);^{1}H-NMR (400MHz , DMSO - d_6 ,δ / *ppm*) : 5.6 (s,2H, CH_2),6.56–7.87(s,8H,Ar-H),4.28(q,2H,-O-CH_2-CH_3),1.32(t,3H,-CH_2-CH_3);^{13}C-NMR(100MHz, DMSO,δ/*ppm*):111.2-152.5(Ar-C),60.3(CH_2), 166.8(CO),61.4(O-CH_2),14.1 (CH_3) .

Synthesis of 4-(1H) - benzotriazolyl methyl amino benzoyl hydrazide (4)

4-(1H) - benzotriazoyl methyl amino benzoate (3) (0.05mole) was refluxed with hydrazine (0.05mole) in absolute ethanol for 10 to12 hours. It was cooled and kept overnight. The solid so obtained was filtered and recrystallized from ethanol. M.P.152–3ºC , yield 46%.(Found : C 56.57 % , H 4.60 % , N 32.84 %; Calcd. C 56.75 % ,H 4.76 % ,N 33.09 %).

IR spectra: 3030-3090 cm^{-1}(C-H of Ar.),1640-1489cm^{-1}(C-C of Ar.),1705–1778(CONH),3450-3550cm^{-1} (-NH-,NH_2)1375–1350 cm^{-1} (-CN); ^{1}H-NMR(400MHz,DMSO-d_6,δ/*ppm*)5.6(s,2H, CH_2),6.56-7.96(s,8H,Ar-H),3.95(2H,s,NH_2),9.65 (1H,s,CONH);^{13}CNMR(100MHz,DMSO,δ/*ppm*) :111.2–153.4(Ar-C),60.3(CH_2),166.6(CO).

Synthesis of Ethyl-2-substituted phenyl hydrazono-3-oxobutyrate (6a–h)

Substituted aniline (5a-h) (0.01mole) was dissolved in HCl (8ml) and water (6ml) mixture. It was cooled to 0ºc in ice bath. A cold aqueous solution of sodium nitrate (0.03mole) was added. The diazonium salt solution was filtered into a cooled solution of ethyl acetate (0.01mole) and sodium acetate (0.12mole), dissolved in ethanol (50ml). The resulting solid was washed with water and recrystallized from EtOH/MeOH. Yields, melting points and other compound characterization data are given in Table 1.

Synthesis of 1-(4-((1H)-benzotriazolyl methyl amino benzoyl)-3-methyl-4-(2-substituted phenyl hydrazono)-1H-pyrazoline-5(4H)-one (7a–h).

A solution of 4-(1H)- benzotriazolyl methyl amino benzoyl hydrazide (0.002mole) in 25 ml of glacial acetic acid was added to Ethyl-2-substituted phenyl hydrazono-3-oxobutyrate (6a–h) (0.002 mole), dissolved in glacial acetic acid (20ml), and

Table 2. Physical and analytical data of the synthesized (7a-h) compounds.

Compound No.	R	Molecular Formula	M.P. ˚C	Yield %	Elemental Analysis C% Found	C% Calcd.	H% Found	H% Calcd.	N% Found	N% Calcd.	Additional ^{1}HNMR Signals (δ , ppm)
7a	H	$C_{24}H_{20}N_8O_2$	208	48	63.68	63.71	4.40	4.42	24.68	24.77	6.48-7.02(s,5H,ArH) 0.9(s,3H,CH_3)
7b	2-Me	$C_{25}H_{22}N_8O_2$	210	53	64.34	64.37	4.69	4.72	23.97	24.03	6.47-7.02 (s,4H,ArH)
7c	4-Cl	$C_{24}H_{19}N_8O_2Cl$	228	46	59.22	59.25	3.87	3.90	22.98	23.04	6.42-7.03 (s,4H,ArH)
7d	4-Br	$C_{24}H_{19}N_8O_2Br$	248	59	54.20	54.23	3.55	3.57	21.02	21.09	6.48-7.02 (s,4H,ArH)
7e	4-NO_2	$C_{24}H_{19}N_9O_4$	196	41	47.26	47.29	3.08	3.11	20.59	20.68	6.72-7.96 (s,4H,ArH)
7f	2,4-Dinitro	$C_{24}H_{18}N_{10}O_4$	214	42	56.45	56.47	3.49	3.52	27.36	27.45	6.98-8.89 (s,3H,ArH)
7g	2,4-Dichloro-6- Nitro	$C_{24}H_{17}N_{11}O_4Cl_2$	192	43	48.53	48.56	2.84	2.86	25.82	25.96	7.22-7.75 (s,2H,ArH)
7h	2,4,6-Tribromo	$C_{24}H_{17}N_8O_2Br_3$	252	54	41.78	41.79	2.44	2.46	15.22	16.25	7.14 (s,2H,ArH)

this mixture was refluxed for 10–12 hrs. It was then cooled and allowed to stand overnight. The resulting solid was filtered off, dried, and crystallized from methanol. Yields, melting points, and other compound characterization data are given in Table 2.

BIOLOGICAL SCREENING

Antibacterial activities

The antibacterial activities of all compounds in 50 μg/ml concentration were studied against gram-positive bacteria *(Bacillus subtilis and Staphylococcus aurous*) and gram-negative Bacteria (*E. coil, Salmonella typhi and Klebsiella promioe*) using the agar cup plate method. This method uses a methanol system for control. We carried out a control experiment under similar conditions, using tetracycline as a standard for a comparison. The area of zone inhibition was measured in mm. An examination of the data reveals that all compounds showed good antibacterial activity. The results are presented in Table 3.

Antifungal activity

The fungicidal activity of all synthesized compounds was studied at 1000 ppm concentration in vitro of plant pathogenic organisms, listed in Table 4. The antifungal activities of all the samples were measured by cup plate method [12–14]. Each of the plant pathogenic strains in potato dextrose agar (PDA) medium. Such a PDA medium contained 200 g of potato, 20g of dextrose, 20 g of agar, and 1 litre of water; 5 day old cultures were employed. The compounds to be tested were suspended (1000 ppm) in a PDA medium and autoclaved at 120º C for 15 min at 15 atm pressure.

Table 3. Antibacterial activity of the (7a-h) compounds.

Compound No.	Zone of Inhibition: Gram-positive *Bacillus subtilis*	Gram-positive *Staphylococcus aureus*	Gram-negative *Klebsiella promioe*	Gram-negative *Salmonella typhl*	Gram-negative *E.coil*
7a	54	57	46	44	63
7b	43	65	57	54	67
7c	72	79	83	76	57
7d	82	69	70	67	83
7e	43	63	67	42	87
7f	64	59	44	64	37
7g	65	53	71	67	56
7h	82	65	58	76	71
Tetracycline	79	55	84	73	72

Table 4. Antifungal activity of the (7a-h) compounds.

Compound No.	Zone of Inhibition at 1000 ppm (%): *Penicillium expansum*	*Botrydepladia thiobromine*	*Nigrospora sp.*	*Trichothesium sp.*	*Rhizopus nigricuns*
7a	74	62	70	53	49
7b	62	73	64	54	67
7c	71	73	78	67	63
7d	67	69	68	79	71
7e	54	57	74	62	69
7f	51	64	58	73	58
7g	61	73	62	58	51
7h	73	78	62	74	67

These medium were poured into sterile Petri plate, and the organisms were inoculated after cooling the Petri plate. The percentage inhabitation for fungi was calculated after 5 days using the formula given below:

PERCENTAGE OF INHIBITION =100 (X-Y)/X

Where, X: Area of colony in the control plate

Y: Area of colony in the test plate

The fungicidal activity of all compounds is shown in Table 4.

RESULTS AND DISCUSSIONS

The synthesis of (6a-h) has been performed based on the method reported. Only (6 b-d) has been reported [1]. The 4-(1H) - benzotriazolyl methyl amino benzoyl hydrazide (4) compound has been synthesized successfully as Mannich reaction, as reported previously [2]. It was observed that ethyl-2-substituted phenyl hydrazono-3-oxobutyrate (6a-h) condensation with 4-(1H) - benzotriazolyl methyl amino benzoyl hydrazide (4) gives 1-(4-((1H)-benzotriazolyl methyl amino benzoyl)-3-methyl-4-(2-substituted phenyl hydrazono) -1H-pyrazoline-5(4H)-one (7a-h).

The structures of (6a-h) were confirmed by elemental analysis and IR spectra, showing an absorption band at 3030–3080 cm^{-1} (C-H of Ar.), 3320-3250 (NH) , 2825–2910 (CH aliphatic) , 1295-1100 (C-O), 1610–1570(C=N), 2950, 1370 cm^{-1} (-CH_3,CH_2), 1720–1660 (C=O).

^{1}H-NMR (400MHz , DMSO - d_6 ,δ / *ppm*) : 1.29(3H,t,CH_3), 2.25 (3H,s,$COCH_3$), 4.25 (2H,q,$COCH_2$), 11.62 (1H,s,NH); (2a): 6.46–7.02 (s,5H,ArH); (2b): 2.36 (s,3H,CH_3),6.34-6.83 (s,4H,ArH) ; (2c): 6.4–7.03 (s,4H,ArH); (2d): 6.35–7.20 (s,4H,ArH); (2e): 6.71–7.94 (s,4H,ArH) ; (2f): 6.98-8.38 (s,3H,ArH); (2g): 7.42–7.36 (s,2H,ArH); (2h): 7.30-7.32 (s,2H,ArH) (s,2H,ArH).

^{13}C-NMR(100MHz,DMSO,δ/*ppm*):14.2(CH_3), 62.6(OCH_2),27.1(CH_3), 163.5-196.4 (-CO), 126.9 (C=N); (2a): 114.6–143.7 (Ar-C); (2b): 17.9 (CH_3), 113.4–142.1 (Ar-C); (2c): 118.2-130.4(Ar-C); (2d): 116.9-142.5(Ar-C),(2e)113.7-149.5(Ar-C);(2f) 116.9-139.1(Ar-C); (2g) 125.1–140.8(Ar-C);(2h) 109.8 -152.7(Ar-C). The C, H, N analysis data of all compounds are given in Table 1.

The IR spectra of all (7a-h) compounds show prominent spectral band due to 3270–3220 (NH),2825–2910(CH aliphatic),1770–1640 (C=O, Amide C=O),1610–1570(C=NNH),3030–3088cm^{-1} (C-H of Ar.).

^{1}H-NMR (400MHz , DMSO - d_6 ,δ / *ppm*) : 2.42(3H,s,CH_3), 5.6 (1H,s,CH_2),9.4–11.56 (2H,s, NH), 6.87–8.04 (complex,m,8H ,ArH); (4a): 6.48-7.02(s,5H,ArH); (4b):0.9(s,3H,CH_3)6.47–7.02 (s, 4H, ArH) ; (4c): 6.42–7.03 (s,4H, ArH); (4d): 6.48–7.02 (s,4H,ArH) ; (4e): 6.72–7.96 (s,4H,ArH); (4f): 6.98–8.89 (s,3H,ArH); (4g): 7.22-7.75 (s,2H,ArH); (4h): 7.14 (s,2H,ArH).

^{13}C-NMR(100MHz,DMSO,δ/*ppm*):11.6 (CH_3) , 60.2–61.4 (CH_2) , 163.7–172.4 (CO) , 129.4–149.5 (C=N); (4a): 112.2–149.7 (Ar-C); (4b): 17.5 (CH_3), 112.2-149.6 (Ar-C); (4c): 112.9–149.4 (Ar-C); (4d): 112.9–149.5(Ar-C), (4e) 112.4–149.9 (Ar-C); (4f)112.1–149.2(Ar-C);(4g)112.3–149.8(Ar-C);(4h)110.7-149.4(Ar-C).The C, H, N analysis data of all compounds are given in Table 2.

The examination of data reveals that the elemental content is in consistence with the predicted structure, shown in Scheme-1. The IR and NMR spectral data also direct for assignment of the predicted structure. The LC- MS of one sample, i.e.7a, shows the peak of M^+ ion at 451.5, which is consistent of molecular weight of 7a, i.e. 448.5. All these facts confirm the (7a-h) structures.

The examination of antibacterial activity data reveals that the compounds 7c, 7e and 7h are found to be more active against gram-positive and gram-negative bacteria.

CONCLUSION

In this paper, we report on the synthesis, the elemental analysis, the spectral studies, and the evaluation of antimicrobial activity of 1-(4-((1H)-benzotriazolyl methyl amino benzoyl)-3-methyl-4-(2-substituted phenyl hydrazono)-1H-pyrazoline-5(4H)-one (7a –h). The antimicrobial activity of these noval compounds was carried out against some strain bacteria. The results show that the synthesized compounds were toxic against the bacteria and fungus. The comparison between the antibacterial and antifungal activity of these compounds and the standard drugs shows that the presence of halogen groups in the phenyl ring increases the antimicrobial activity. In conclusion, we report herein, a simple and convenient route for the synthesis of some new heterocyclic compounds, based on pyrazolinone, which are of antimicrobial action.

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Синтеза и анти-бактериална активност на някои нови азо-пиразоли

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(Резюме)

Установено е, че реакцията на Маних на бензо-триазол (1), етил-4-амино бензоат (2) и формалдехид в етанол позволява получаването на 4-(1H)-бензотиазоил-метил-амино-бензоат (3), който при третирането с хидразин-хидрат в присъствие на етанол дава 4-(1H)-бензотриазоил-метил-амино-бензоил хидразид (4). Това съединение е получено чрез кондензация на различни предварително приготвени етил-2-субституирани фенил-хидразоно-3-оксо-бутирати (6a-h) и дава 1-4-(1H)-бензотриазоил-метил-амино-бензоил)-3-метил-4-(заместен фенилхидразоно)-2-пиразолин-5-он (7a-h). Всички съединения (7a-h) са охарактеризирани с IR and NMR спектроскопия. Тези съединения показват значителна анти-микробна активност срещу различни бактерии и гъбички.