Int. J. Chem. Sci.: 14(4), 2016, 2799-2808
ISSN 0972-768X
www.sadgurupublications.com

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF N'-(2-HYDROXY-5-(ARYLAZO) BENZYLIDENE-5-(BENZOFURAN-2-YL)-1-PHENYL-1H-PYRAZOLE-3-CARBOHYDRAZIDE DERIVATIVES

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ABSTRACT

A straight forward one pot two component synthesis of seven novel pyrazole based carbohydrazone derivatives has been described. Prominent feature of this synthetic process is a condensation of 5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (**2)** with 2-hydroxy-5-(arylazo)benzaldehyde (**1a-g)** to afford directly N'-(2-hydroxy-5-(arylazo) benzylidene–5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide derivatives (**3a-g)**. The structural identities of carbohydrazone derivatives were confirmed by elemental analysis and spectral studies such as FT-IR, ^{1}H NMR and Mass spectroscopy. The inhibitory effect of newly synthesized compound **3e** was investigated *in-vitro* against Gram +ve and Gram -ve bacteria at different concentrations. **3e** showed good to moderate inhibitory effects compared with standard drug Chloramphenicol. It was inactive against *S. aureus* and *E. areogenes* at a lower concentration of 31 µg/mL, while at rest of all the concentrations it showed inhibitory zone against used bacterial strains.

Key words: Carbohydrazone, Pyrazole, Benzofuran, m-Arylazo salicylaldehyde.

INTRODUCTION

Heterocyclic moieties make up a massively important class of compounds and have great value in many applications such as, medicinal, pharmaceutical, agrochemical, functional materials, among many others. Among them pyrazole made indispensable anchor for design and development of new pharmacological agents possessing a wide spectrum of biological activities like, antibacterial and antifungal[1], anticancer[2], antiviral[3], antiimflamatory[4], antitubercular[5], antagonists of the CB_1 receptor[6], enzyme inhibitor agents[7], anticorrosion activity[8] etc.

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Compounds containing general formula of ArCONHN=C(R/Ar) are known as N-acyl hydrazones. Hydrazones contains an azomethine CO-NH-N=CH group which plays a vital role in the biological activities like antimicrobial[9], anticancer[10], anti-inflammatory[11], antiparasitic[12]. Hydrazones are well known intermediate for the synthesis of azetidinones, thiazolidinones, oxadiazolines and many other derivatives. They are generally prepared by reacting acid hydrazides with aldehydes or ketones either in solvent free condition or in various solvents. Literature survey reveals that common method for preparation of the N-acyl hydrazones involves the treatment of substituted acid hydrazides and carbonyl compounds in suitable solvents such as ethanol, 1,4-Dioxane, DMF, in presence of acidic catalyst such as HCl, AcOH, heteropolyacids or piperidine as basic catalyst in ethanol.

Moreover, azo compounds are important structure in the medicinal, pharmaceutical and chemistry of dyes. Azo dyes containing heterocyclic ring have been studied widely due to their excellent medicinal properties, such as antibacterial[13], antiviral[14], anti-fungal[15], antioxidant activities[16] along with thermal[17] and optical activity[18]. They are still very important for applications such as disperse dyes for polyester fibers[19-20]. Rizk and co-workers synthesized azo reactive dyes having pyrazole moiety and studied their fastness properties, colour assessment, antibacterial and antifungal activity[21], also Dabholkar and co-workers synthesized pyrazole derivative containing azo dye moiety and studied their antimicrobial study[22].

As part of our recent efforts to develop new pyrazole based carbohydrazone derivatives connected with azo link we thought to synthesize the carbohydrazone derivatives by one pot two component method. Combination of the pyrazole, azomethine and azo moiety may enhance their therapeutic value thus, simultaneously carried out their antibacterial activities.

EXPERIMENTAL

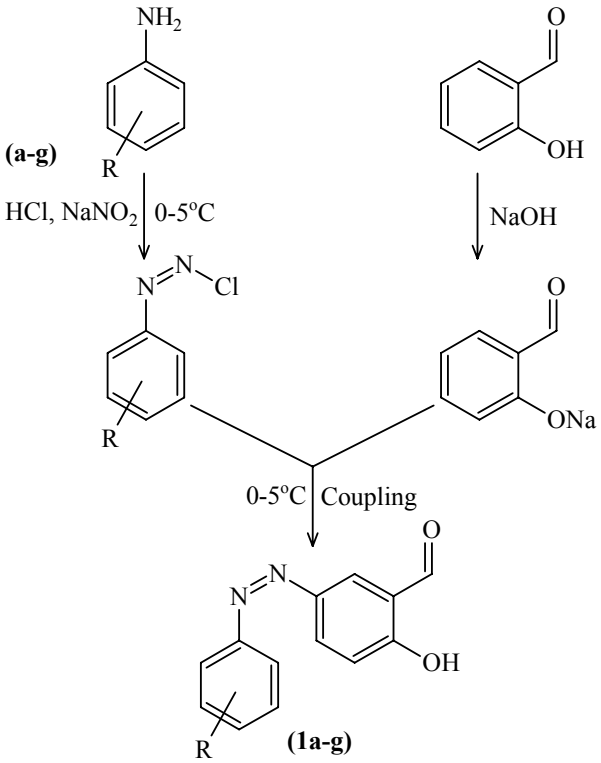
Materials and methods

All solvents were of commercial quality used from freshly opened containers and were dried and purified by conventional methods and were purchased from Merck, S.D. Fine and Aldrich. The melting points were recorded in open capillary in paraffin bath and are uncorrected. ^{1}H NMR spectra are recorded on a Bruker AM 400 instrument (400 MHz) using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 as solvent. Chemical shifts are given in parts per million (ppm). IR spectra were recorded on a Shimadzu. IR Spectrophotometer (KBr, v max in cm^{-1}). Positive-ion Electro Spray Ionization (ESI) mass spectra were obtained with a Waters Micromass Q–TOF Micro, Mass Spectrophotometer.

Elemental (CHN) analysis was done using Thermo Scientific (Flash-2000). The compounds were analysed for carbon, hydrogen, nitrogen and the results obtained are in good agreement with the calculated values. The reactions were monitored by E. Merck TLC aluminium sheet silica $gel_{60}F_{254}$ and visualizing the spot in UV Cabinet and iodine chamber.

Procedure for the synthesis of 2-hydroxy-5-(arylazo) benzaldehyde (1a-g)

p-Toluidine (**a**, 9 g, 0.084 mol) was dissolved in hydrochloric acid (6 M, 50 mL) and diazotized using sodium nitrite (8 g, 0.115 mol) maintaining the temperature 0-5°. A cold solution of salicylaldehyde (10 g, 8.55 mL, 0.0819) in aqueous sodium hydroxide (2N, 80 mL) was added slowly with continuous stirring to the diazotized solution. The resulting dark orange solid was washed with water and recrystallized from acetic acid to give **1a**. Similarly, **1b-g** were synthesised from different substituted aniline **(a-g)** by extending the same procedure followed for **1a** (**Scheme 1**).



Entry	a	b	c	d	e	f	g
R	4-CH_3	4-Br	4-Cl	2-OEt	4-OEt	3-OMe	4-OMe

Scheme 1

2-hydroxy-5-(p-tolylazo) benzaldehyde (1a)

Yellow crystalline solid; mp,154-156^{o}C; yield 94%; M. F. $C_{14}H_{12}O_2N_2$; IR (KBr v max in cm^{-1}): 3185 (-OH), 3030, 2994 (ArH),1719, 1655 (C=O), 2742 (C-H in CHO) 1479, 1500 (C=C), 1454 (N=N); ^{1}H NMR(DMSO-d_6) δ ppm: 2.40 (s, 3H, Ar-CH_3), 10.3790 (s, 1H, OH), 11.4137 (s, 1H, CHO), 7.1859-8.1835 (m, 7H, ArH); MS: *m/z* 241 $[M+H]^+$, 242 $[M+2]^+$. Elemental Anal.Calcd: for $C_{14}H_{12}O_2N_2$; C, 69.99; H, 5.03; N, 11.66; Found: C, 70.12; H, 4.94; N, 11.13. **1b:** Yellow crystalline solid; mp,160-162^{o}C; yield 79%; M. F. $C_{13}H_9BrO_2N_2$; **1c:** Yellow amorphous; mp,159-161^{o}C; yield 77%; M. F. $C_{13}H_9ClO_2N_2$; **1d:** Orange crystalline solid; mp,140-142^{o}C; yield 77%; M. F. $C_{15}H_{14}N_2O_3$; **1e:** Dark brown crystalline solid; mp,144^{o}C; yield 76%; M. F. $C_{15}H_{14}N_2O_3$; **1f:** Dark brown crystalline solid; mp, 98-100^{o}C; yield 75%; M. F. $C_{14}H_{12}N_2O_3$ **1g:** Dark brown crystalline solid; mp,110-112^{o}C; yield 77%; M. F. $C_{14}H_{12}N_2O_3$.

General procedure for the synthesis of N'-(2-hydroxy-5-(arylazo) benzylidene-5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (3a-g)

A mixture of 2-hydroxy-5-(p-tolylazo)benzaldehyde (**1a**, 0.01 mol**)** and 5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (**2**, 0.01 mol**)** in ethanol in presence of 2-3 drops of acetic acid was refluxed for 2-3 h and the completion of reaction was monitored by TLC, the reaction mixture was allowed to cool, filtered and recrystallized from 1,4-Dioxane to give **3a**.

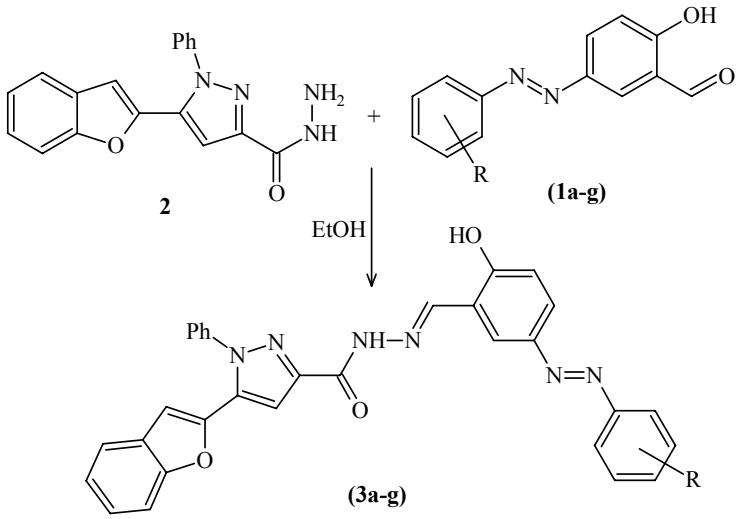
Similarly, **3b-g** were synthesised from **1b-g** and **2** by extending the same procedure followed for **3a** (**Scheme 2**).

N'-(2-hydroxy-5-(P-tolylazo) benzylidene)-5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (3a)

Orange crystalline solid; recrystallization solvent, 1,4-Dioxane; mp, 258-260^{o}C; yield, 85%. IR (KBr v max in cm^{-1}): 3788 (O-H), 3380, 3337 (-NH), 1665 (C=O), 1490 (C=C), 1275, 1225, 1083 (C-O-C), 1432 (N=N), 1613 (C=N, Pyrazole). Elemental anal. Calcd: for $C_{32}H_{24}N_6O_3$; N, 15.55 Found: N, 15.23.

N'-(2-hydroxy-5-(4-bromophenylazo) benzylidene)-5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (3b)

Orange crystalline solid; recrystallization solvent, 1,4-Dioxane; mp, 276-278^{o}C; yield, 79%; IR (KBr v max in cm^{-1}): 3790 (O-H), 3420, 3323 (-NH), 3040 (Ar-H), 1667 (C=O), 1490 (C=C), 1275, 1225, 1083 (C-O-C), 1428 (N=N), 1546 (C=N), 1613 (C=N, Pyrazole). Elemental Anal. Calcd: for $C_{31}H_{21}BrN_6O_3$; N, 13.88 Found: N, 13.37.



Entry	a	b	c	d	e	f	g
R	4-CH_3	4-Br	4-Cl	2-OEt	4-OEt	3-OMe	4-OMe

Scheme 2

N'-(2-hydroxy-5-(4-chlorophenylazo) benzylidene)-5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (3c)

Orange crystalline solid; recrystallization solvent, 1,4-Dioxane; mp, 272-273^{o}C; yield, 77%; IR (KBr v max in cm^{-1}): 3760 (O-H), 3314, 3240 (-NH), 3030 (Ar-H), 1655 (C=O), 1448, 1490 (C=C), 1215, 1089 (C-O-C), 1433 (N=N), 1537 (C=N), 1606 (C=N, Pyrazole). Elemental Anal. Calcd: for $C_{31}H_{21}ClN_6O_3$; N, 14.98 Found: N, 14.73.

N'-(2-hydroxy-5-(2-ethoxyphenylazo) benzylidene)-5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (3d)

Orange crystalline solid; recrystallization solvent, 1,4-Dioxane; mp, 218-221^{o}C; yield, 80%; IR (KBr v max in cm^{-1}): 3792 (O-H), 3420, 3323 (-NH), 3020 (Ar-H), 1666 (C=O), 1487 (C=C), 1275, 1271, 1100 (C-O-C), 1429 (N=N), 1533 (C=N), 1610 (C=N, Pyrazole). Elemental Anal. Calcd: for $C_{33}H_{26}N_6O_4$; N, 14.73 Found: N, 14.43.

N'-(2-hydroxy-5-(4-ethoxyphenylazo) benzylidene)-5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (3e)

Yellow solid; solvent for recrystallization, 1,4-Dioxane; mp, 212-214^{o}C; yield, 82%; M. F. $C_{33}H_{26}N_6O_4$; IR (KBr v max in cm^{-1}): 3832 (O-H), 3409, 3223, 3152 (-NH), 3063 (Ar-

H), 1677 (C=O), 1599, 1500, 1474 (C=C), 1242, 1042 (C-O-C), 1552 (C=N), 1577 (C=N, Pyrazole) 1414 (N=N); ^{1}H NMR: 4.13-4.15 (q, 2H, O-CH_2-CH_3), 1.35-1.39 (triplet, 3H, O-CH_2-CH_3), 6.60 (s, 1H, pyrazole at C_4 Position) 8.31 (s, 1H, N=CH), 8..85 (s, 1H, NH) 7.09-8.12 (m, 18H, ArH & -OH); MS: *m/z* 571 $[M+H]^+$, 572 $[M+2]^+$, 593 $[M+Na]^+$, 594 $[(M+1)+Na]^+$**.** Elemental Anal. Calcd: for $C_{33}H_{26}N_6O_4$; C, 69.46; H, 4.59; N, 14.73 Found: C, 69.11; H, 4.34; N, 14.27.

N'-(2-hydroxy-5-(3-methoxyphenylazo) benzylidene)-5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (3f)

Orange crystalline solid; recrystallization solvent, 1,4-Dioxane; M.F. $C_{32}H_{24}N_6O_4$; mp, 220-222°C; yield, 75%; IR (KBr v max in cm^{-1}): 3783 (O-H), 3442, 3367 (-NH), 3045 (Ar-H), 1695 (C=O), 1486 (C=C), 1259, 1097 (C-O-C), 1430 (N=N), 1534 (C=N), 1602 (C=N, Pyrazole). Elemental anal. Calcd: for $C_{32}H_{24}N_6O_4$; N, 15.10 Found: N, 14.02.

N'-(2-hydroxy-5-(4-methoxyphenylazo) benzylidene)-5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide (3g)

Orange crystalline solid; recrystallization solvent, 1,4-Dioxane; mp, 240-242°C; yield; 78%; IR (KBr v max in cm^{-1}): 3765 (O-H), 3456, 3352 (-NH), 3050 (Ar-H), 1673 (C=O), 1488 (C=C), 1239, 1055 (C-O-C), 1428 (N=N), 1527(C=N),1609 (C=N, Pyrazole). Elemental anal. Calcd: for $C_{32}H_{24}N_6O_4$; N, 15.10 Found: N, 14.94.

Antibacterial activity

The novel synthesized heterocyclic compounds **3e** was screened for its *in-vitro* antimicrobial activity using cup plate agar disc-diffusion method against two Gram positive bacterial strains, *B. thurengienesis* and *S. aureus* and two Gram negative strains, *E. coli* and **.** *E. areogenes.* Chloramphenicol was used as standard drug.

General procedure: Determination of zone of inhibition by agar disc-diffusion method

Test solutions were prepared with known weight of compound in DMSO and half diluted suitably to give the resultant concentration of 31-1000 µg/mL. Whatmann no. 1 sterile filter paper discs (6 mm) were impregnated with solution and allowed to dry at room temperature. *In vitro* antibacterial activity was determined by using Mueller Hinton Agar obtained from Himedia Ltd., Mumbai. Petri plates were prepared by pouring 10 mL of Mueller Hinton Agar for bacteria containing microbial culture was allowed to solidify. The discs were then applied and the plates were incubated at 37°C for 24 h (bacteria) and the

inhibition zone was measured as diameter in four directions and expressed as mean. The results were compared using Chloramphenicol as a standard antibacterial agent. The results of antibacterial activity (i.e. zone of inhibition in mm) are given in the Table 1.

RESULTS AND DISCUSSION

In the present paper, a novel series of pyrazole based carbohydrazone derivatives linked with azo moiety were synthesized. At every stage purity of the compound were monitored by TLC technique. The structure of newly synthesized compound **3e** has been established on the basis of its elemental and spectral analysis such as IR, ^{1}H NMR and Mass and other synthesised compounds have also been characterised by IR and nitrogen estimation.

Reaction sequences employed for the synthesis of starting compounds by coupling reaction of diazotized product of aromatic primary amines (**a-g)** with salicylaldehyde afforded **1a-g** is shown in scheme **1**. The IR spectrum of **1a** showed band due to aromatic stretch (C-H and C=C) in expected region and bands appear in the region 1655, 2742, 3185, and 1454 cm^{-1} were attributed to the C=O and C-H stretch in –CHO group, O-H in –OH group and N=N stretching in azo group respectively. The ^{1}H NMR spectrum of **1a** showed singlet at δ 2.40 ppm for –CH_3 group, hence it confirms that diazonium salt of p-toluidine has coupled with sodium salt of salicylaldehyde to afford (E)-2-hydroxy-5-(arylazo) benzaldehyde **1a-g.**

The compound **2** (5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide) was also achieved in quantitative yield by the reference method[23]. The reaction of 2-hydroxy-5-(arylazo) benzaldehyde (**1a-g)** with 5-(benzofuran-2-yl)-1-phenyl-1H-pyrazole-3-carbohydrazide **(2)** in ethanol as a solvent afforded **3a-g,** is described in the reaction Scheme **2**.

The IR spectrum of compound **3e** reveals vibration at 3063 cm^{-1} over the range corresponding to Ar-H and Het-H vibration stretch. The strong absorption band at 1677 cm^{-1} is attributed to C=O stretching in -CONHN=C group of pyrazole carbohydrazide. Further the absorption peak due to -OH stretching appears at 3832 cm^{-1} and also exhibited bands due to NH stretching at 3409, 3223, 3152 cm^{-1}. Further confirmation comes out from its ^{1}H NMR spectrum which showed expected signals for aromatic and aliphatic proton. which also showed singlet at δ 8.85 ppm for -NH proton and a singlet at δ 8.31 ppm for N=CH proton. Also each singlet appeared for CH_2 and CH_3 protons at δ 4.13 and δ 1.35 ppm, respectively.

According above data confirms that 5-((4-ethoxyphenyl)azo)-2-hydroxybenzaldehyde **(1e)** condenses with **2** to get final compound **3e**. Its elemental analysis reveals that % of C, H, N are 69.11, 4.34, 14.27 and respectively, while its mass spectrum shows a molecular ion peak at *m/z* 571 $[M+H]^+$ matches with the molecular formula $C_{33}H_{26}O_4N_6$.

Antibacterial activity

Data obtained from antibacterial assessment and furnished in Table 1 indicates that the test compound **3e** showed good antibacterial activity against Gram positive bacteria, *B. thurengienesis* and *S. aureus.* But at the concentration of 31 µg/mL it was found to be inactive against *S. aureus.* In case of gram negative bacteria, **3e** showed moderate activity against *E.coli* and *E. aerogenes* and it is inactive against *E. aerogenes* at concentration of 31 µg/mL. On the basis of data it is clear that pyrazole based carbohydrazone derivatives possesses antibacterial activity.

Table 1: Antibacterial activity of 3e

S. No.	Conc. (µg/mL)	Zone of inhibition (mm)			
		Gram +ve		Gram -ve	
		B. thurengienesis	***S. aureus***	***E. coli***	***E. aerogenes***
		3e			
1	1000	7	15	10	10
2	500	14	14	12	10
3	250	15	16	13	8
4	125	8	10	12	10
5	63	12	8	13	8
6	31	8	-	14	-
		Standard chloramphenicol			
1	1000	22	26	24	16
2	500	20	30	20	16
3	250	21	27	18	17
4	125	16	21	17	16
5	63	15	18	17	15
6	31	16	20	21	15

CONCLUSION

A series of novel carbohydrazone derivatives **(3a-g)** were successfully synthesized in good yields. Their purity and confirmation was checked by physical, analytical and spectral data. Antibacterial screening of **3e** compounds was found to possess moderate activity against selected strains of bacteria also found it is inactive at lower concentration and rest of synthesised will be assessed in future study.

ACKNOWLEDGEMENT

The authors are thankful to The Principal, Government Science College, Gadchiroli, for his support and cooperation. The authors are also thankful to Dr. S. D. Narkhede, Head, Department of Botany, GSC, Gadchiroli for permitting to carry out the antimicrobial activity, similarly the authors are also thankful to The Director, SAIF, Punjab University, Chandigarh for providing CHN analysis, IR, ^{1}H NMR and Mass Spectra.

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Accepted : 03.11.2016