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Synthesis and Antimicrobial Activity Some Novel N-[3-(Substituted Aryl)-1-Phenyl -1h-Pyrazol-4 Yl] Methylene Triazole Derivatives

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Abstract

Schiff Base derivatives are important class of compounds. They possess different types of Biological activities like antibacterial, antiviral, anti HIV, antifungal etc. Schiff base derivatives are prepared by the condensation of aldehyde and amine and these compounds are characterized by chemical and instrumental methods. Their important biological properties have been investigated.

Key words: Schiff Base derivatives, Biological study, trizole derivatives, Hydrazone derivatives.

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INRODUCTION

Hydrazones, possessing an azomethine –NHN=CH- proton, constitute an important class of compounds for new drug development. Therefore, many researchers have synthesized these compounds as target structures and evaluated their biological activities. Hydrazones are synthesized by heating the appropriate substituted hydrazine /hydrazides with aldehydes and ketones in solvents like ethanol, methanol, butanol, glacial acetic acid, ethanol-glacial aceticacid[1].These are well known intermediates for the preparation of oxadiazolines [2], azetidinones [3], thiazolidinones [4] and many other derivatives. Hydrazones exhibit a wide range of pharmacological activities like Anticancer [5], Ant malaria [6], and Ant tubercular [7] etc.

A large number of substituted pyrazole derivatives are prepared and tested for variety of biological activities like Anti HIV[8], Antiviral[9], Ant parasitic[10]and Fungicidal [11]etc.

1,2,4-Triazole is a planner five member heterocyclic system with two carbon and three nitrogen atoms(one pyrazole type and two pyridine type) in the 1,2,4 positions. It was also named as v-triazole (v for vicinal) to distinguish it from s- triazole[12](s for symmetrical), 1,2,4 triazole exists in two taut metric forms, 1H and 2H forms in which 1H form was initially considered more stable than the 2H form but spectral studies have confirmed the predominance of symmetrical 2H form.

RESULTS AND DISCUSSION

The synthesis of N-((1,3-diphenyl-1H-pyrzol-4yl)methylene) -4H-1,2,4-triazol-3-amine derivatives (Ia-m) involved the reaction between appropriate 1, 3 –diphenyl- 1H-pyrazole- 4-carbaldehyde (B_{a-m}**)** and 4-amino 1,2,4 tri azole as described in the general procedure.

IR spectra showed the N-H stretching vibration peak at 3399.72 cm^{-1} and The Schiff base also confirmed by an intense band of C=N around 1675.46cm^{-1}. The other peaks of IR spectra also prove the structure of hydrazones derivatives. The nuclear magnetic resonance spectra (^{1}H NMR) showed the hydrazied (N-H) proton as a singlet at 8.5423 ppm and the amine proton (N=C-H) at 8.5423 ppm and the mass spectrum of comp. (II-a) shows the $[M+1]^+$ quasi molecular ion ^{p} (m/z = 315) a base peak. Many times, due to collision of secondary ion with sample quasi molecular ion, $[M+1]^+$ or $[m+2]^+$ is formed and is sometimes prominent base peak, which undergoes less fragmentation.As per the nitrogen rule, it must have even molecular weight, which is 314.34 (isotopic mass). 316 peak is 20.40 % of 315 $[M+1]^+$ peak indicating the presence of 18 carbon atoms (confirmed by the rule of thirteen). Fragments showed peaks at m/z 160 [(M+1) - 155] and m/z etc.

Antimicrobial activity

Antimicrobial activity testing was carried out by using Agar cup method. Each purified compound was dissolved in dimethyl sulfoxide (DMSO), sterilized by filtration using sintered glass filter and stored at 4^0C. All the synthesized compounds were screened for their antibacterial and antifungal activities against the E. coli, P. auregenosa, S. aures, S. pyogenus and the fungi C. albicans, A. niger, and A. clavatus. The compounds were tested at 250, 100, 50 and 25 concentration using nutrient agar tubes. The highest dilution showing at least 99 % inhibition is taken as MBC (minimal bactericidal concentration). Control experiments were carried out under similar condition by using gentamycine, ampicillin and chloramphenicol for antibacterial activity and nystatin and greseofulvin for antifungal activity as standard drugs.

The antibacterial activity data are given in Table – 2b, which clearly show that the compound ii-c, ii-d, and ii-e having a Hydroxyl substitute in o, p, & m position of the phenyl ring exhibited signification activity against E. coli, P. auregneosa, S. aures and S. pyogenus. Compound ii-d showed equal antibacterial activity as amplicilline (against S. aureus) and compound ii-c showed equal antibacterial activity as chloramphenicol (against S. pyogenes). Compounds ii-c and ii-d had an almost similar range of MBC value (against E.coli and P.auregenosa). They differ only by two or three fold dilution, in spite of having a hydroxyl substituent at different positions. The replacement of the hydroxyl substituent by a nitro (-NO_2) or a methoxy (-OCH_3) or a chloro (-CI) group in phenyl ring as ii-f, ii-g, ii-l, ii-b and ii-k respectively, caused a reduction of activity. Compound ii-j showed equal antibacterial activity as amplicilline (against P. aeruginosa).

Table no. 2a: physical and chemical characteristics of n-((1, 3-diphenyl-1H-pyrzol-4yl) methylene) -4H-1, 2, 4-triazol-3-amine

Compound No.	R	Molecular formula.	Formula Weight.	Solvent for crystallization (final Step)	% yield Colour	M.P. C R.F	% Carbon Found (Calcu.)	% Hydrogen Found (Calcu.)	% Nitrogen Found (Calcu.)
II-a	$-C_6H_5$	$C_{18}H_{14}N_6$	314.34	Ethanol	84/w	144/0.63	68.65/(68.71)	4.40/(4.48)	26.85/(26.72)
II-b	4-Cl-C_6H_4	$C_{18}H_{13}N_6Cl$	348.33	Ethanol	80/w	127/0.56	61.60/(61.65)	3.70/(3.73)	23.90/(23.98)
II-c	2-OH-C_6H_4	$C_{18}H_{14}N_6O$	330.33	Ethanol	89/y	160/0.66	65.30/(65.38)	4.27/(4.27)	25.40/(25.43)
II-d	4-OH- C_6H_4	$C_{18}H_{14}N_6O$	330.33	Ethanol	87/y	110/0.61	65.35/(65.38)	4.25/(4.27)	25.42/(25.43)
II-e	3-OH- C_6H_4	$C_{18}H_{14}N_6O$	330.33	Ethanol	84/y	225/0.69	65.34/(65.38)	4.21/(4.27)	25.45/(25.43)
II-f	4-NO_2- C_6H_4	$C_{18}H_{13}N_7O_2$	359.93	Ethanol	82/y	117/0.74	60.15/(60.14)	3.55/(3.64)	27.21/(27.29)
II-g	3-NO_2- C_6H_4	$C_{18}H_{13}N_7O_2$	359.93	Ethanol	88/y	122/0.64	60.09/(60.14)	3.57/(3.64)	27.24/(27.29)
II-h	4-Br- C_6H_4	$C_{18}H_{13}N_6Br$	392.12	Ethanol	81/y	148/0.66	55.01/(55.08)	3.30/(3.34)	21.35/(21.42)
II-i	4-CH_3SO_2- C_6H_4	$C_{19}H_{16}N_6O_2S$	392.13	Ethanol	85/w	167/0.70	58.08/(58.14)	4.05/(4.11)	21.31/(21.40)
II-j	2,4diOH- C_6H_4	$C_{18}H_{14}N_6O_2$	346.11	Ethanol	84/y	160/0.62	62.31/(62.40)	4.11/(4.17)	24.21/(24.27)
II-k	2,4diCl- C_6H_4	$C_{18}H_{12}N_6Cl_2$	386.11	Ethanol	83/w	230/0.61	55.85/(55.94)	3.08/(3.13)	21.71/(21.76)
II-l	4-OCH_3- C_6H_4	$C_{19}H_{16}N_6O$	344.13	Ethanol	88/y	170/0.63	66.20/(66.25)	4.60/(4.68)	24.35/(24.41)
II –m	4-CH_3- C_6H_4	$C_{19}H_{16}N_6$	328.14	Ethanol	81/w	105/0.63	69.40/(69.48)	4.85/(4.91)	25.50/(25.60)

w=white,y=yellow

Table No.:- 2b Antibacterial Activity Table
Minimal Bacterial Concentration

COM.	CODE	E. COLI MTCC 443					P.AERUGINOSA MTCC 424					S AUREUS MTCC 96					S .PYOGENES MTCC 442				
NO	R	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
II-a	C_6H_5	-	14	16	17	18	-	10	13	15	19	-	13	16	17	19	-	11	14	15	18
II-b	4-Cl-C_6H_4	-	11	15	18	19	-	12	13	15	17	-	12	14	15	17	-	13	16	17	19
II-c	2-OH- C_6H_4	-	13	14	19	21	-	11	13	14	16	-	11	16	18	20	-	12	15	17	21
II-d	4-OH-C_6H_4	-	14	15	18	22	-	10	11	14	16	-	12	14	15	16	-	12	14	15	19
II-e	3-OH-C_6H_4	-	12	14	17	21	-	12	15	17	20	-	11	13	15	16	-	9	13	15	19
II-f	4-NO_2-C_6H_4	-	14	15	17	19	-	10	14	16	18	-	15	18	19	20	-	11	12	15	16
II-g	3-NO_2-C_6H_4	-	13	14	15	16	-	11	12	13	15	-	10	13	15	18	-	12	13	15	21
II-h	4-Br-C_6H_4	-	12	13	15	19	-	11	13	15	17	-	10	15	16	18	-	12	15	18	21
II-i	4-CH_3SO_2-C_6H_4	-	13	17	18	19	-	9	11	12	13	-	12	13	15	17	-	12	14	18	21
II-j	2,4di-OH-C_6H_3	-	15	17	18	18	-	14	16	18	19	-	15	16	17	19	-	13	14	14	18
II-k	2,4di-Cl-C_6H_3	-	12	15	19	23	-	10	13	18	21	-	12	15	16	18	-	13	15	17	20
II-l	4-OCH_3- C_6H_4	-	15	18	18	22	-	12	18	19	21	-	11	14	18	20	-	12	15	18	20
II-m	4-CH_3- C_6H_4	-	15	13	17	18	-	12	12	13	14	-	12	15	17	19	-	12	16	18	21

Table No.:- 2c

Minimal Bacterial Concentration

Standard Drugs	**E-Coli MTCC 443**					**P.AERUGINOSA MTCC 424**					**S. Aureus MTCC 96**					**S.PYOGENES MTCC 442**				
	5 µg /ml	25 µg /ml	50 µg /ml	100 µg/ ml	250 µg/ ml	5 µg /ml	25 µg/ ml	50 µg/ ml	100 µg/ ml	250 µg/ ml	5 µg/ ml	25 µg /ml	50 µg /ml	100 µg/ ml	250 µg/ ml	5 µg/ ml	25 µg/ ml	50 µg/ ml	100 µg/ ml	250 µg/ ml
Amplicilline	14	15	16	19	20	14	15	15	18	20	10	13	14	16	18	11	14	16	18	19
Chloramphenicol	14	17	23	23	23	14	17	18	19	21	12	14	19	20	21	10	13	19	20	20
Ciprofloxacin	20	23	28	28	28	20	23	24	26	27	17	19	21	22	22	16	19	21	21	22
Norfloxacin	22	25	26	27	29	18	19	21	23	23	19	22	25	26	28	18	19	20	21	21

µg/ml =micro gram /ml
5, 25, 50,100,250 =Various Concentration

Table No.:- 2d Antifungal Activity Table

Minimal Fungicidal Concentration

COMP.	**CODE**	**A. NIGER MTCC 282**					**C. ALBICANS MTCC 227**				
NO	R	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
II-a	C_6H_5	-	22	25	25	25	-	18	18	19	22
II-b	4-Cl- C_6H_4	-	20	22	25	27	-	21	21	23	24
II-c	2-OH- C_6H_4	-	19	21	23	25	-	18	20	24	25
II-d	4-OH- C_6H_4	-	19	22	25	25	-	19	21	22	25
II-e	3-OH- C_6H_4	-	18	19	22	25	-	18	20	22	22
II-f	4-NO_2- C_6H_4	-	19	21	24	25	-	19	22	25	25
II-g	3-NO_2- C_6H_4	-	18	20	23	25	-	18	18	21	22
II-h	4-Br- C_6H_4	-	21	22	23	25	-	21	22	23	25
II-i	4-CH_3SO_2- C_6H_4	-	18	20	22	23	-	18	19	21	22
II-j	2,4di-OH- C_6H_3	-	20	22	23	25	-	22	22	24	24
II-k	2,4di-Cl- C_6H_3	-	19	21	23	23	-	18	20	21	22
II-l	4-OCH_3- C_6H_4	-	20	21	23	25	-	20	22	25	25
II-m	4-CH_3- C_6H_4	-	18	21	24	24	-	19	22	24	25

The antifungal activity data in Table – 2d clearly show that the compound ii-h having a Bromo substitute in a p- position of the phenyl ring exhibited significant activity against C .albicans and A. niger. Compound ii-h showed equal antifungal activity as greseofulvin (against C.albicans) and some less activity against A. Niger. Compounds ii-b and ii-j showed equal antifungal activity as greseofulvin (against C.albicans) but high activity against A. Niger.

Spectral study of N-[(1, 3 –diphenyl-1H-pyrazol-4-yl) methylene- 4H-(1, 2, 4 triazol- 3-amine (ii-a) [isotopic weight = 314.34 g].
IR (KBr) cm^{-1}: 1675.46 (C=N Stretching of Schiff base), 2946.85 (C-H Str. Asym.), 1355.66 (C-H def. sym.), 3032.84 (Ar C-H Stretching), 1595.96 (C=N Str. Of pyrazole ring), 1520.20 (C=N triazole moiety) and 1392.76 (C-N triazole moiety).; **H NMR ($CDCI_3$) δ (ppm):** 10.505 (1 H, s –NH-), 9.3430 (1 H, s –CH=N-), 8.5423 (1H, s pyrazol ring), 7.2554-7.8367 (11 H, Ar-H); **Mass Spectra (m/z)** = 315 $(M+1)^+$, 316(M+2), 180, 160, 142.

Table No.:- 2e Minimal Fungicidal Concentration

Standard Drugs	**A. Niger MTCC 282**					**C. Albicans MTCC 227**				
	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml
Greseofulvin	19	23	25	25	28	18	21	22	22	24
Nystatin	18	19	24	29	29	18	21	24	25	26

µg/ml =micro gram /ml,
5, 25, 50,100,250 =Various Concentration

MATERIALS AND METHODS

The compounds N-[(1, 3 –diphenyl-1H-pyrazol-4-yl) methylene- 4H-(1, 2, 4 triazol- 3-amine (II_{a-m}) were obtained by following preparation method (Ia) [Figur-1]

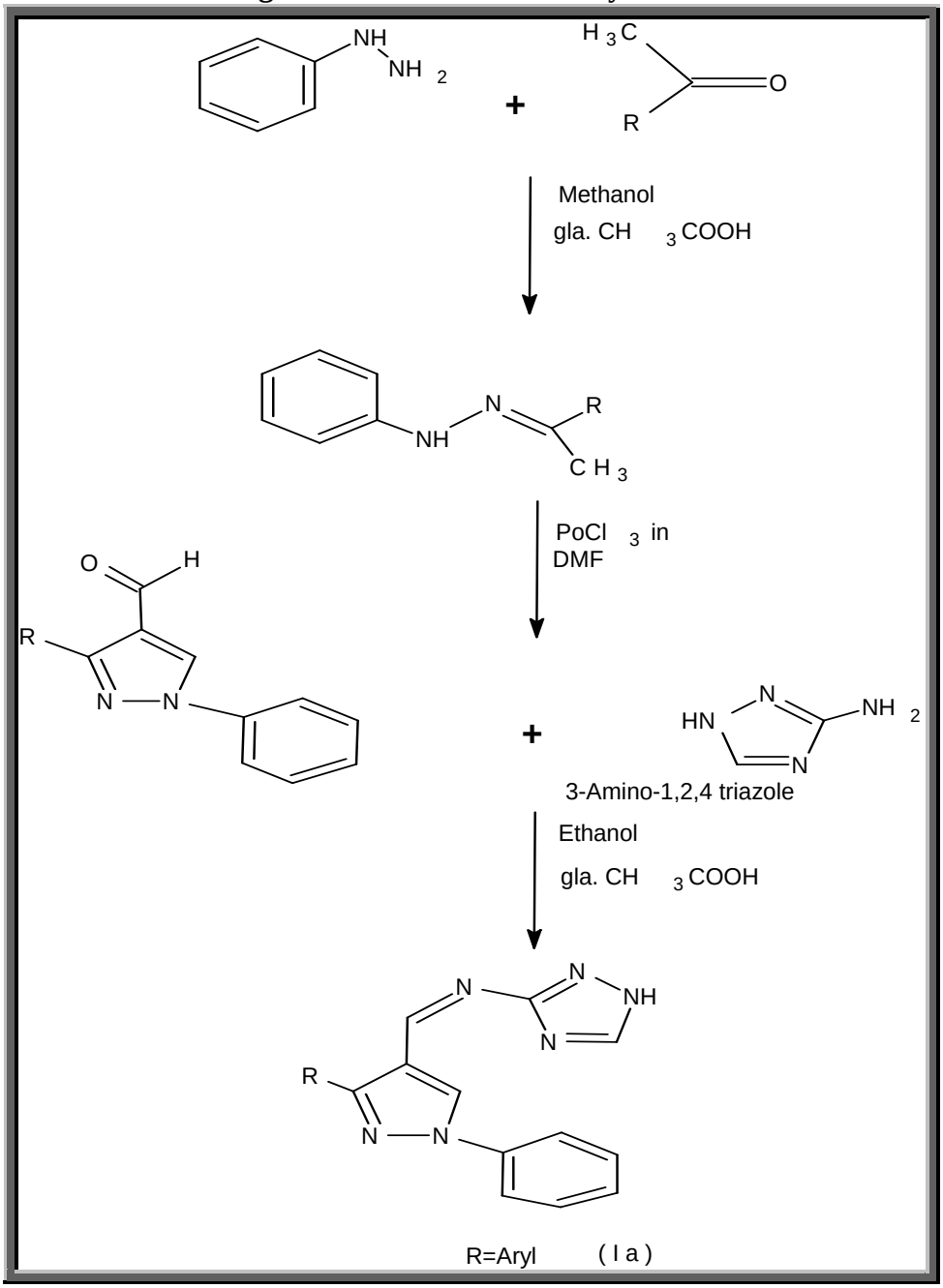
[A] Synthesis of N-Phenyl amino-α-methyl-phenyl azomethine
A mixture of phenyl hydrazine (1.08gm, 0.01M) and acetophenone (1.20gm, 0.01M) in absolute ethanol was refluxed in water bath for 4 hrs. In presence of 1ml glacial acetic acid. Product obtained after cooling was crystallized from absolute ethanol. Yield, 1.8gm (90%), M.P.: 64°C. ($C_{14}H_{14}N_2$; Calculated: C, 80.00; H, 6.66; N, 13.37%; Found: C, 79.92; H, 6.64; N, 13.34%).

This typical experimental procedure was followed to prepare other analogs of this series.

[B] Synthesis of 1, 3 –diphenyl- 1H-pyrazole- 4- carbaldehyde
N-Phenyl amino-α-methyl-phenyl azomethine (0.84gm, 0.004M) was added in a mixture of Vilsmeier – Hack reagent (prepared by drop wise addition of 1.2ml $POCl_3$ in ice cooled 10ml DMF) and refluxed for 6hrs. The reaction mixture was poured into crushed ice followed by neutralization using sodium bicarbonate. Crude product was isolated and crystallized from

methanol. Yield, 2.16gm (87%), M.P.: 120°C. ($C_{16}H_{12}N_2O$; Calculated: C, 77.42; H, 4.48; N, 11.29%; Found: C, 77.39; H, 4.80; N, 11.28%).
Exactly similar experimental procedure was followed to prepare other analogs of this series.

Figure 1 General Process of synthesis



[C] N-[(1, 3 –diphenyl-1H-pyrazol-4-yl) methylene- 4H-(1, 2, 4 triazol- 3-amine)
A mixture of 1, 3- diphenyl-1H- pyrazole-4- carbaladehyde (2.48gm, 0.01M) and 3-Amino 1,2,4-triazol (0.84gm,0.01M) was taken in absolute ethanol and few drops of glacial acetic acid was

added. Then the mixture was refluxed for 6 h on water bath. The separated solid was filtered, washed and recrystalized from ethanol.
M.P. 144°C, Yield 84%, and $C_{18}H_{14}N_6$; Calculated: C, 68.71; H, 4.48; N, 26.72; Found: C, 68.65; H, 4.40; N, 26.85%)

The same experimental procedure was utilized to prepare other analogs of this series **(Ia-m).** The purity of synthesized compounds was checked by thin layer chromatography (TLC) on silica gel plate using ethyl acetate: cyclohexane (80: 20). Melting points were determined by open capillary method and are uncorrected. IR spectra are recorded on FT-IR Perkin-Elmer spectrophotometer RXI using KBr disc. ^{1}H NMR spectra are recorded on in $CDCL_3$ ON a Bruker DRX-400 MHz using TMS as inter standard. The chemical shifts are reported as parts per million (ppms) and ESI MS were determined on Discovery Make Thermo Spectrometer.
The characterization data of compounds (Ia-m) are described in Table 2a and antimicrobial data are described in Table 2b, 2c, 2d and 2e.

CONCLUSION

Thirteen pyrazole derivatives were synthesized and characterized for their possible structure. Spectra and chemical analyses supported the expected structural formula. These compounds were subjected to antibacterial and antifungal screening. The antibacterial and antifungal activities were less compared to the standard drugs.

However, certain structural alterations did not increase antimicrobial activity and working ahead in that direction may give quite promising results.

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