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Synthesis and Biological study of Substituted 1, 2, 4-Triazolines and their acetyl derivatives.

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ABSTRACT

In the reaction of isoniazid with different aldehydes the respective N^1-((un)-substituted benzilidene) isoniazid derivatives were obtained. Further reaction with N^1-((un)-substituted benzilidene) isoniazids and hydrazine hydrate yielded dihydroformazans. These dihydroformazans on reaction with N-phenylisocynodichloride in chloroform medium led to the creation of 1, 2, 4-triazolines. All these 1,2,4-triazolines were further converted into their acetyl derivatives by reacting them with acetic anhydride. The structures of all new compounds were confirmed by using elemental and spectral analysis. All the compounds were screened for their antifungal activities.

Keywords: 1, 2, 4-Triazolines, Dihydroformazans, Synthesis of 1, 2, 4-triazolines.

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INTRODUCTION

1, 2, 4-Triazole and its derivatives represent one of the most biologically active class of compounds possessing a wide spectrum of activities. Several five membered heterocyclic systems have three heteroatoms at symmetrical positions have been studied because of their interesting physiological studies. The 1,2,4- triazole nucleus is related with various pharmacological activities such as antimicrobial, fungicidal, anti-inflammatory, antiparasitic, insecticidal, herbicidal, antiviral, antitumor, anticonvulsant, antidepressant, hypotensive effects and plant growth regulatory activities[1-5] .It is also well established that various derivatives of 1, 2, 4-triazoles exhibit broad spectrum pharmacological properties. The literature survey revealed that 1, 2, 4-triazole are prepared by different routes[6-13]. The present work describes the synthesis of new 1, 2, 4-triazolines heterocyclic bases by unknown route.

MATERIAL AND METHODS

All the reagents used in this synthesis were of analytical grade and used without further purification. The melting points were determined in open capillaries and are uncorrected. The infrared spectrum was recorded on PerkinElmer instrument. ^{1}H NMR spectra was recorded in DMSOd6/$CDCl_3$ using TMS as an internal standard. The chemical shifts are expressed in □ ppm. The completion of reactions were observed using TLC.

Preparation of N-phenyl isocynodichloride:

This was prepared by following known procedure[14].

Preparation of N^1-((un)-substituted benzilidene/ethylidene) isoniazid:

These were synthesized by the following known method of Sacconi[15]. The typical synthesis where (R= Ethylidene) is described in details.

Preparation of N^1-(ethylidene) isoniazid (IIa):

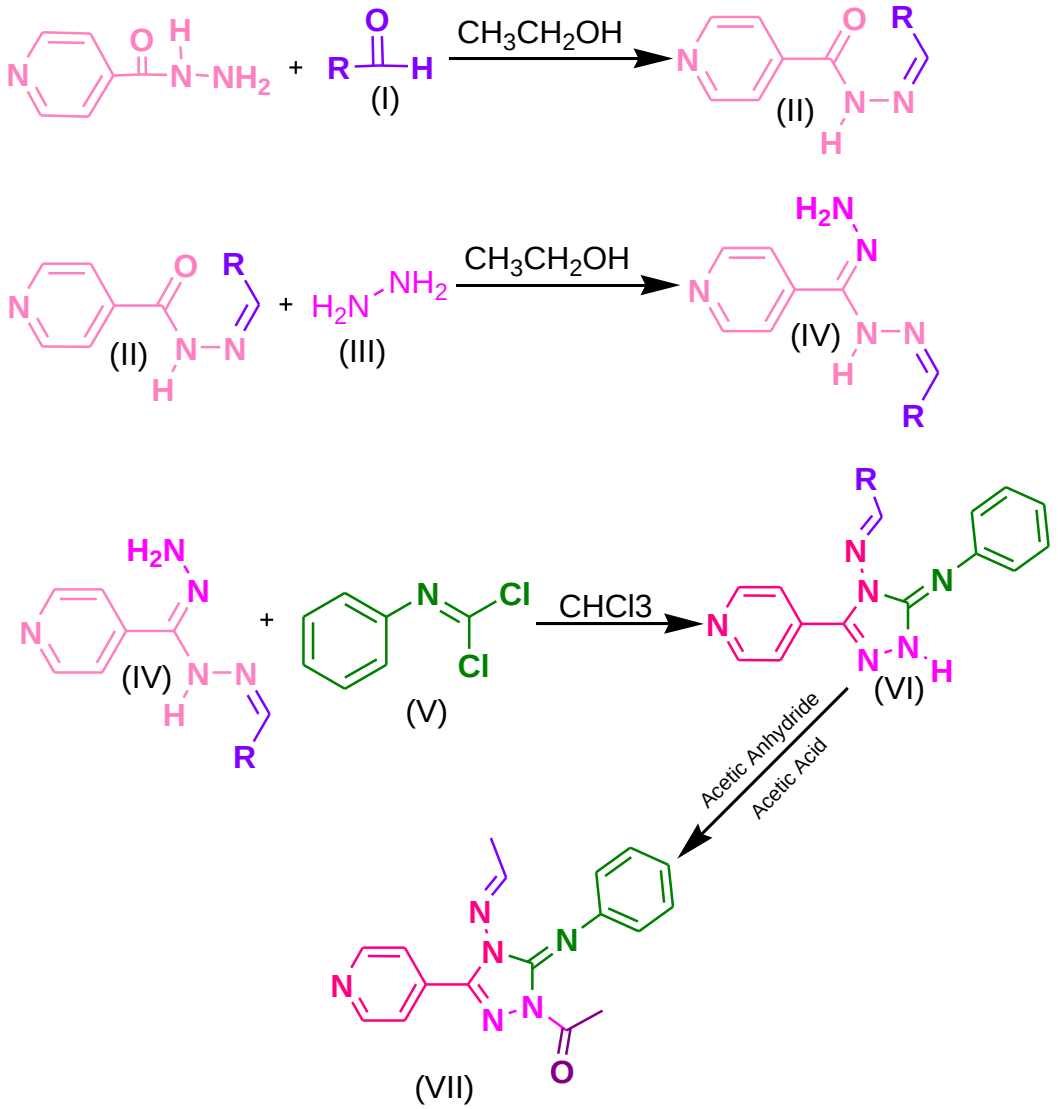
Isoniazid (0.01mole) was added in acetaldehyde (Ia) (0.01mole) and 20ml ethanol. The reaction mixture was refluxed for 2 hours under water bath. On distilling off the solvent, solid was separated out, it was crystallized from ethanol.

Compound (IIb – IIh) were synthesized by the extension of above method.

Synthesis of N^1- (ethylidene)-3-(pyrid-4yl)-dihydroformazan(IVa):

N^1-(ethylidene) isoniazid (IIa) (0.01 mole), hydrazine hydrate (III) (0.01 mole) and 20ml ethanol as solvent were refluxed for 2 hours under water bath. After completion of the reaction the solvent was distilled off N1- (ethylidene)-3-(pyrid-4yl)-dihydroformazan (IVa) was separated out. It was crystallized from ethanol.

By extending the above reaction related dihydroformazans are (IVb – IVh) isolated.



Reaction Scheme

Synthesis of 3-(pyrid-4yl)-4-(2-hydroxy benzilidene) amino-5-phenylimino-1, 2, 4-triazoline (VIa):

To a chloroform suspension of N^1-(2-hydroxy benzilidene-3-(pyrid-4yl)-dihydroformazan (IVa) (0.01 mole) was added a chloroform solution of N-phenyl isocynodichloride (0.01 mole) and the mixture was refluxed over water bath for 3 hours. Evolution of hydrogen chloride gas was noticed. After completion of the reaction solvent was distilled off when solid compound (VIa) was isolated. It was washed several times with petroleum ether (60 – 80^O) followed by ethanol. The compound was crystallized from ethanol. On extending the above reaction the other 1,2,4-triazolines were isolated.

Synthesis of 1-acetyl- 3-(pyrid-4yl)-4-(4-methoxy benzilidene) amino-5-phenylimino-1,2,4-triazoline (VIIa):

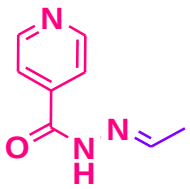
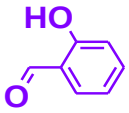
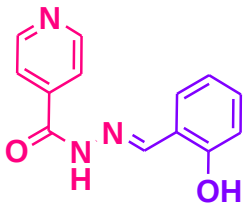
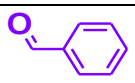
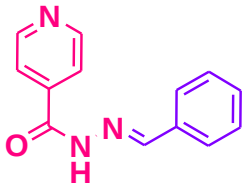
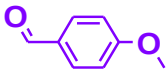
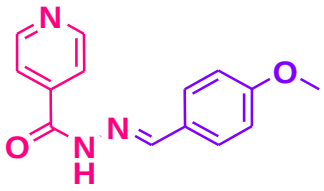
3-(pyrid-4yl)-4-(4-methoxy benzilidene) amino-5-phenylimino-1,2,4-triazoline (VIc) (0.01 mole), acetic anhydride (0.01 mole) and glacil acetic acid were refluxed together for 1 hour followed by dilution with water, solid (VIIa) was separated out. It was washed with water, crystallized with aqueous ethanol. Compound (VIIb – VIIh) were synthesized by the extension of above method.

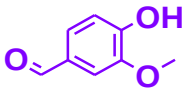
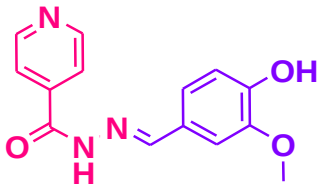
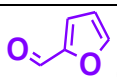
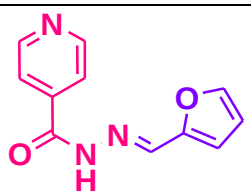
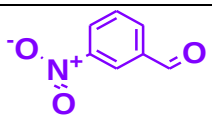
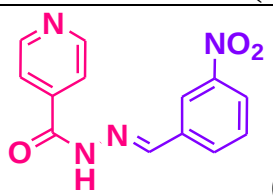
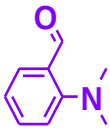
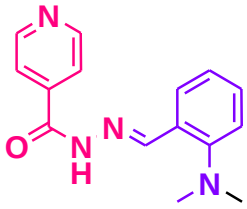
RESULTS AND DISCUSSION

Preparation of N^1-(ethylidene) isoniazid (IIa):

Melting point of the compound was observed as 176 ^{O}C, yield 85%. The major absorption bands observed in IR spectrum[16-17] of the product are listed below ν (N-H) 3303, ν (C=N) 1667, ν (C=O) 1634, ν (C-N) 1334, ν (N-N) 1220. (Found: C 58.70, H 5.40, N 25.00%, $C_8H_9N_3O$; requires C 58.90, H 5.52, N 25.77%). Compound (IIb – IIh) shows good yield, (Table-1).

Table 1: Formation of N^1-(substituted benzylidene)isoniazid(II) Reagent: Ioniazid and Aryl/alkyl aldehyde(I)

Aryl/alkyl aldehyde(I)	N^1-(substituted benzylidene)isoniazid(II)	Yield (%)	Melting Point (^{o}C)
 (Ia)	 (IIa)	85	176
 (Ib)	 (IIb)	85	250
 (Ic)	 (IIc)	91	200
 (Id)	 (IId)	90	130

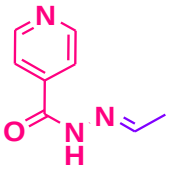
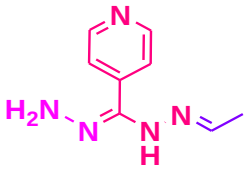
 (Ie)	 (IIe)	89	220
 (If)	 (IIf)	70	225
 (Ig)	 (IIg)	75	210
 (Ih)	 (IIh)	88	170

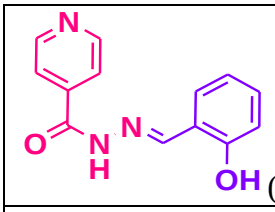
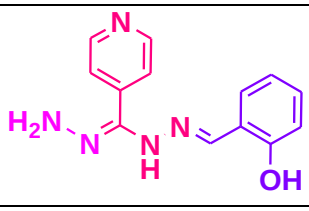
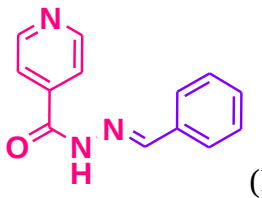
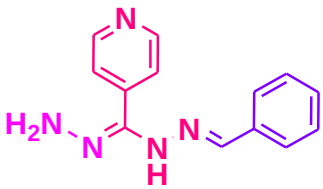
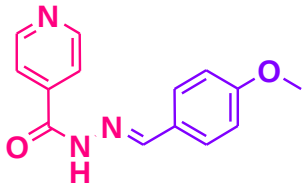
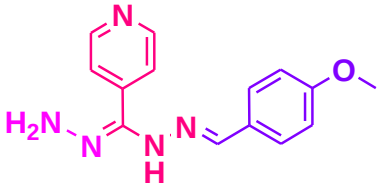
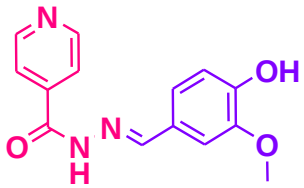
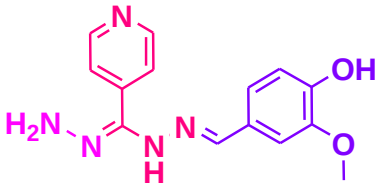
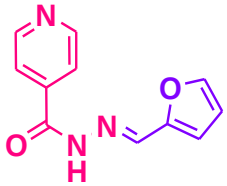
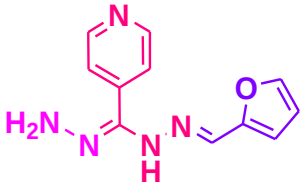
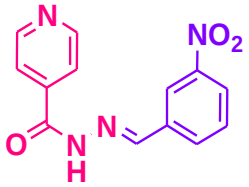
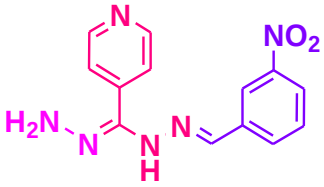
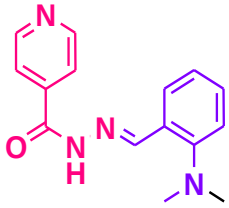
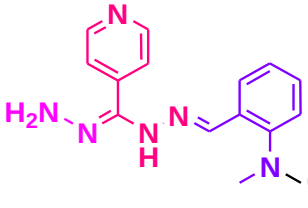
Synthesis of N^1- (ethylidene)-3-(pyrid-4yl)-dihydroformazan(IVa):

Melting point 180 ^{O}C , yield, 90%. The absorption bands observed in the infrared spectrum are: ν (N-H)3304, ν (C=N)1667, ν (C-N)1333, ν (N-N)1219.

The NMR[18-19] spectrum of the compound (IVa) display signals due to the methyl protons at (δ 2.1 ppm), =CH- protons at (δ 2.6 ppm), NH (-NH_2) protons at (δ 5.3 ppm), pyridyl protons at (δ 7.8-8.9 ppm) and NH (-CONH) protons at (δ 9.8 ppm). (Found: C, 52.45; H, 06.10; N, 39.43% $C_8H_{11}N_5$ requires: C, 52.54; H, 06.21; N, 39.54%). Compound (IVb – IVh) shows good yield, (Table-2).

Table 2: Formation of N^1-((Un)substituted benzylidene)-3-(pyrid-4-yl) dihydroformazan(IV) Reagent: N^1-(substituted benzylidene) Ioniazid(II) and Hydrazine Hydrate(III)

N^1-(substituted benzylidene)isoniazid(II)	N^1-((Un)substituted benzylidene)-3-(pyrid-4-yl)-dihydroformazan(IV)	Yield (%)	Melting Point (^{o}C)
 (IIa)	 (IVa)	70	180

 (IIb)	 (IVb)	88	138
 (IIc)	 (IVc)	90	140
 (IId)	 (IVd)	86	150
 (IIe)	 (IVe)	88	110
 (IIf)	 (IVf)	75	158
 (IIg)	 (IVg)	75	115
 (IIh)	 (IVh)	90	182

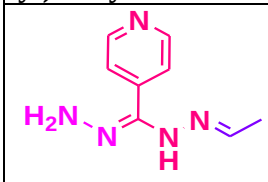
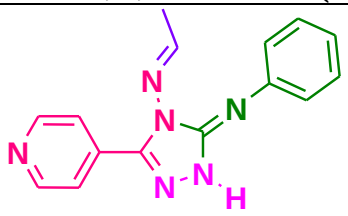
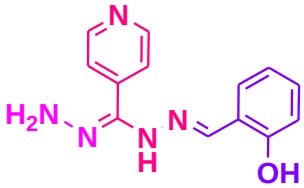
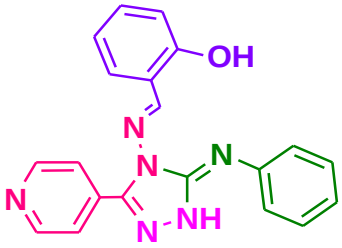
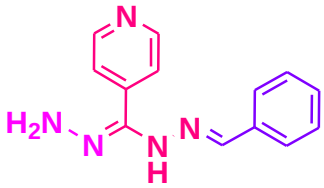
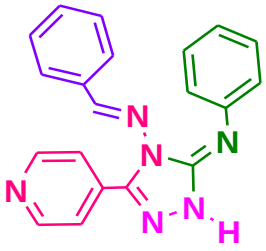
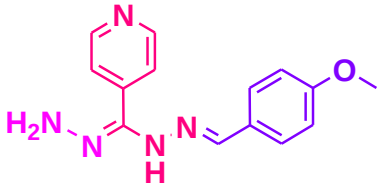
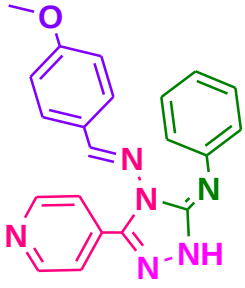
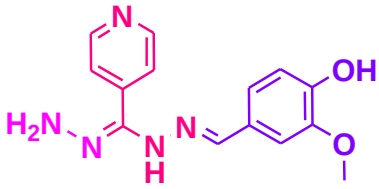
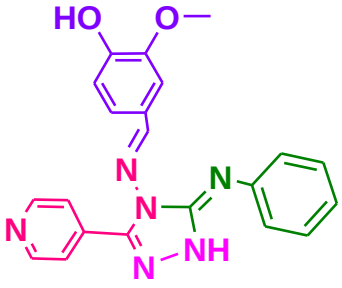
Synthesis of 3-(pyrid-4yl)-4-(2-hydroxy benzilidene) amino-5-phenylimino-1,2,4-triazoline (VIa):

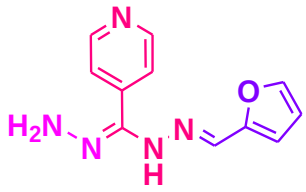
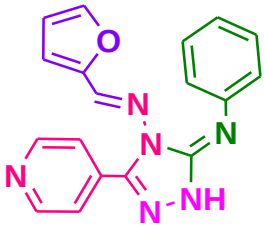
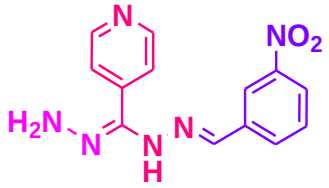
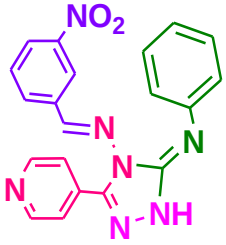
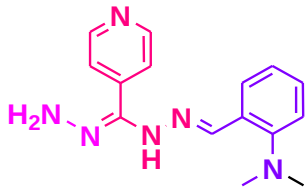
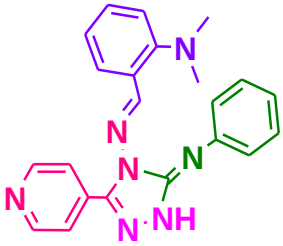
Melting point is 118 ^{O}C, yield 81%. The main absorption bands observed in the infrared spectrum of (VIa) are: δ (O-H)3400, δ (N-H)3200, δ (C=N)1621, δ (C-N)1272, δ (N-N)1196.

The PMR spectrum of (VIa) distinctly displayed signals due to aromatic protons at (δ 6.9 – 7.9 ppm), NH proton at (δ ppm), pyridyl protons at (δ ppm), OH proton at (δ 2.1 ppm) and =CH

proton at (δ 6.8 ppm) respectively. (Found: C, 67.03; H, 4.40; N, 23.49% $C_{20}H_{16}N_6O$ requires C, 67.41; H, 4.40; N, 23.59%). Compound (VIb – VIh) shows good yield, (Table-3).

Table 3: Formation of 3-(pyrid-4-yl)-4-((Un) substituted benzylidene) amino-5-phenyl imino-1, 2, 4-triazoline (VI) Reagent: N^1-((Un)substituted benzylidene)-3-(pyrid-4-yl)-dihydroformazan(IV) and N-phenyl isocyanodichloride(V)

N^1-((Un)substituted benzylidene)-3-(pyrid-4-yl)-dihydroformazan(IV)	3-(pyrid-4-yl)-4-((Un) substituted benzylidene) amino-5-pheny imino-1, 2, 4-triazoline (VI)	Yield (%)	Melting Point (^oC)
 (IVa)	 (VIa)	81	118
 (IVb)	 (VIb)	88	140
 (IVc)	 (VIc)	80	110
 (IVd)	 (VId)	76	150
 (IVe)	 (VIe)	88	130

 (IVf)	 (VIf)	83	190
 (IVg)	 (VIg)	70	110
 (IVh)	 (VIh)	71	120

Synthesis of 1-acetyl- 3-(pyrid-4yl)-4-(4-methoxy benzilidene) amino-5-phenylimino-1,2,4-triazoline (VIIa):

Melting point 140 ^{O}C. The absorption bands observed in the infrared spectrum of (VIIa) are:

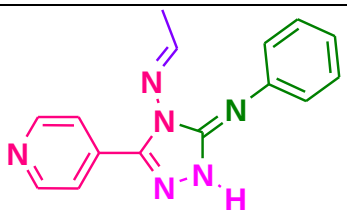
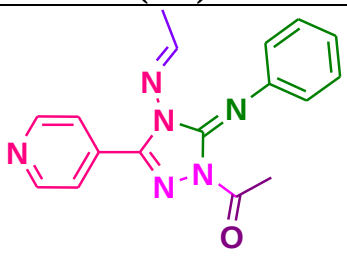
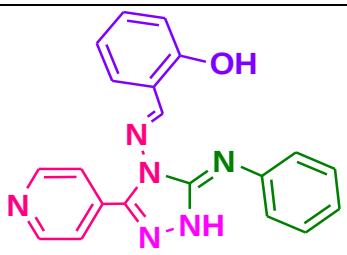
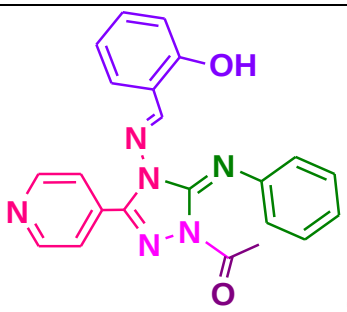
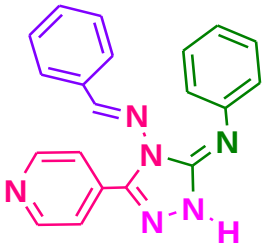
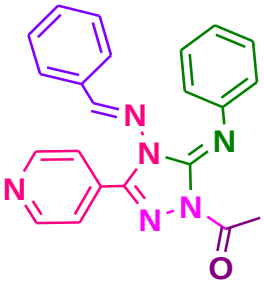
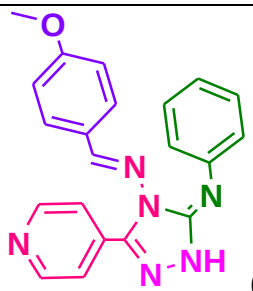
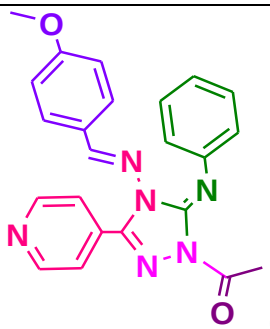
ν (C=O) 1650, ν (C=N) 1580, ν (C-N) 1260, ν (N-N) 1220. The NMR spectrum of (VIIa) display signals due to aromatic protons at (δ 6.9 – 7.8 ppm), NH proton at (δ ppm), pyridyl protons at (δ ppm), $COCH_3$ proton at (δ ppm), COCH3 (δ ppm) and =CH proton at (δ 6.8 ppm) respectively. (Found: C, 66.88; H, 4.80; N, 20.25%, $C_{23}H_{20}N_6O_2$ requires: C, 66.99; H, 4.85; N, 20.38%).

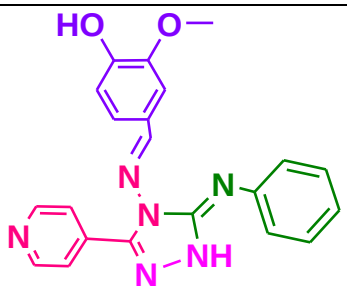
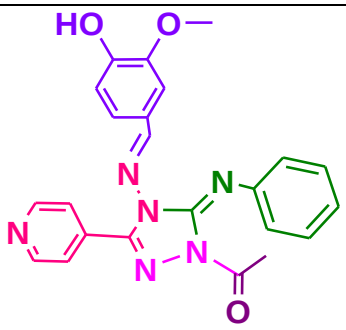
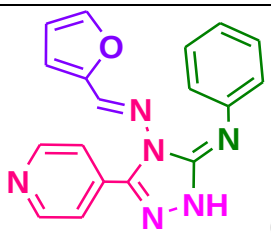
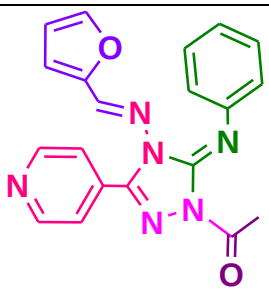
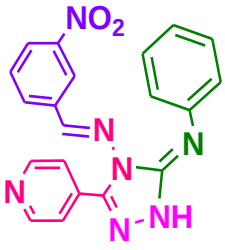
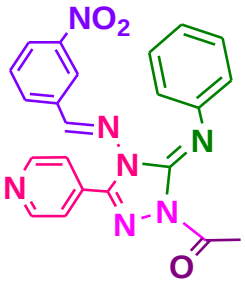
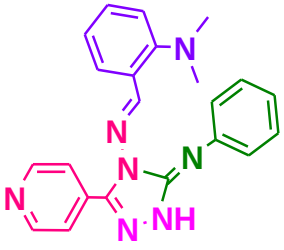
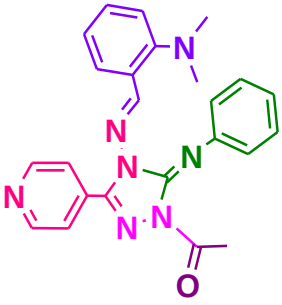
Melting point and structure of the compounds shown in (Table-4). The interaction of N^1-(2-hydroxybenzilidene)-3-(pyrid-4-yl)-dihydroformazan (IVa) and N-phenyl isocynodichloride (V) has been carried out in chloroform medium on boiling water bath for 3 hours, evaluation of hydrogen chloride was clearly noticed. After completion of the reaction and distilling off the chloroform, solid product separated out. It was washed several times with petroleum ether (60-80^{O}) followed by little amount of ethanol. It was crystallized from ethanol to give pure solid, melting point 180 ^{O}C, yield 81%. The elemental analysis of this newly synthesized compound indicated it's molecular formula as $C_{20}H_{16}N_6O$.

The reaction of N-phenyl isocynodichloride was capable of extension to other N^1-((un)-substituted benzilidene)-3-(pyrid-4yl)-dihydroformazan (IVb-IVh) and the corresponding 3-

(pyrid-4yl)-4-((un)substituted benzilidene) amino-5-phenyl imino-1, 2, 4-triazolines (VIb-VIh) have been isolated (Table-3).

Table 4: Formation of 1-acetyl-3-(pyrid-4-yl)-4-((Un) substituted benzylidene) amino-5-Phenyl imino-1, 2, 4-triazoline (VII) Reagent: 3-(pyrid-4-yl)-4-((Un) substituted benzylidene) amino-5-pheny imino-1, 2, 4-triazoline (VI) and Acetic anhyderide

3-(pyrid-4-yl)-4-((Un) substituted benzylidene) amino-5-pheny imino-1, 2, 4-triazoline (VI)	1-acetyl-3-(pyrid-4-yl)-4-((Un) substituted benzylidene) amino-5- Phenyl imino-1, 2, 4-triazoline (VII)	Melting Point (^{o}C)
 (VIa)	 (VIIa)	196
 (VIb)	 (VIIb)	150
 (VIc)	 (VIIc)	140
 (VId)	 (VIId)	172

 (VIe)	 (VIIe)	152
 (VIf)	 (VIIf)	176
 (VIg)	 (VIIg)	130
 (VIh)	 (VIIh)	152

General procedure for acetylation:

3-(pyrid-4yl)-4-((un)substituted benzilidene) amino-5-phenyl imino-1, 2, 4-triazolines (VIa), acetic anhydride and glacial acetic acid were refluxed together for 1 hour followed by dilution with water afforded solid (VIIa), melting point 190 ^{O}C. The elemental analysis of the product indicates the molecular formula as $C_{22}H_{18}N_6O_2$.

The synthesized compounds have done the antibacterial and antifungal activities which are shown in the table.

Biological Activity

All synthesized compounds (VIa-h) have been screened for antifungal activity[20] by using well method. The plates were incubated at 37^{O} C for 24-48 hours. Inhibition zones read after

incubation is over for fungal strains. The compounds were taken at a concentration of 100 µg/ml using DMSO as a solvent. The compounds were screened for their antifungal activity against R. batalicole in nutrient agar medium.

The results of the compounds (VIb,d,e,f,g,h) synthesized given for antifungal screening are mentioned in (Table – 5).

Table 5: Biological activity of 1, 2, 4-triazoline (VI)

Compound	Antifungal activity Zone of inhibition (mm)		%inhibition (mm)		Antifungal activity Zone of inhibition (mm)
	1%	2%	1%	2%	
VIa	***	***	***	***	***
VIb	15.00	59.00	11.36	67.64	08.00
VIc	***	***	***	***	***
VId	10.00	55.00	11.36	62.50	07.00
VIe	30.00	38.00	34.09	43.18	07.00
VIf	31.00	44.00	35.22	50.00	06.00
VIg	28.00	44.00	31.81	58.00	06.00
VIh	25.00	42.00	28.20	47.72	08.00

*** => Activity of the compound not done

CONCLUSION

This study reports the successful synthesis of the title compounds in good yields and shows good antifungal activities. The present method is efficient, mild, simple, convenient and applicable for variety of 1, 2, 4-triazoles.

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REFERENCES

1. Miyauchi H., Kozuki K., Tanio T., Ohashi N. Structureactivity relationships of sulfur containing triazole antifungals. Bioorg. Med. Chem. Lett. 1995; 5(14):1479–1482.
2. Nair H K , Peterson A C , Yazdi P T , Franzmair R. Imidazole and triazole substituted ether phospholipids: Potent antitumor agents. Bioorg. Med. Chem. Lett.1997; 7(18):2379–2382.
3. Wood P M , Woo L W L , Labrosse J , Trusselle M N , Abbate S. New Trisubstituted 1,2,4-Triazole Derivatives as Potent Ghrelin Receptor Antagonists. J. Med Chem.2008; 51:4226–4238.
4. Mavrova A T , Wesselinova D , Tsenov Y A , Pavletta D., Synthesis, cytotoxicity and effects of some 1,2,4-triazole and 1,3,4-thiadiazole derivatives on immunocompetent cells. Eur. J. Med. Chem.2009; 44:63–69.

5. Klimesova V , Zahajska L , Waisser K , Kaustova J ,Mollmann U. Synthesis and antimycobacterial activity of 1,2,4-triazole-3-benzylsulfanyl derivatives. IL. Farmaco.2004; 59:279-288.
6. Wujec M, Stefanska J, Siwek A, Tatarczak M. "Synthesis, Lipophilicity and Antimicrobial Activity of New Derivatives of Thiosemicarbazides and 1,2,4-Triazolines-5- Thione", Acta Poloniae Pharmaceutica - Drug Research, 2009; (66)1:73-82.
7. Pitucha M, Wujec M , Dobosz M , Kosikowska U, Malm A. Synthesis and Biological Action of 1-Aminomethyl Derivatives of 3-R-4-Phenyl-Δ2-1, 2, 4-Triazoline- 5-Thione. Acta Poloniae Pharma Drug Res 2005; (62) 6:443- 449.
8. Derek H, Ji-Hsiung T. Synthesis of C-substituted triazoles, isoxazoles and pyrazoles by 1,3-dopolar cycloaddition to acetylinic sugars. Carbohydr. Res. 1978; (67)2:357-370.
9. Karakuş S , Çoruh U , Barlas-Durgun B , Vázquez-López E M , Özbaş-Turan S , Akbuğa J , Rollas S. Synthesis and cytotoxic activity of some 1,2,4-triazoline-3-thione and 2,5-disubstituted-1,3,4-thiadiazole derivatives. Marmara Pharmaceutical Journal 2010; 14: 84-90.
10. Nepogodiev S A , Dedola S , Marmuse L , de Oliveira M T , Field R A. Synthesis of triazole-linked pseudo-starch fragments. Carbohydr. Res. 2007; 342:529-540.
11. Yasemin U, Esra D, Kemal S, Mustafa E, Alpaykaraoglu S. Synthesis and Antimicrobial Evaluation of Novel Di-triazoles and 4-Arylidene Amino 4,5-Dihydro-1H- [1,2,4] triazole-5-one Derivatives. Turk J Chem.2008; 32: 441 – 455.
12. Mohamed Al-Omar, Al-Abdullah E S, Shehata I A , Habib E E ,Ibrahim T M, Ali A. Synthesis, Antimicrobial, and Anti- inflammatory Activities of Novel 5-(1-Adamantyl)-4-arylideneamino-3-mercapto-1, 2,4triazoles and Related Derivatives. Molecules. 2010; 15: 2526-2550.
13. Khosrow Z , Khalil F , Taraneh T, Mohammad Reza S. Synthesis and Antimicrobial Activity of Some Pyridyl and Naphthyl Substituted 1, 2, 4-Triazole and 1, 3, 4-Thiadiazole Derivatives. Turk J Chem. 2004; 28:95 - 100.
14. Ottmann G, Hooks. Chlorination of Isothiocyanates. 11. N-Aryl- and N-Alkyl- S-chloroisothiocarbamoyl Chlorides, a Novel Class of Sulfenyl Chlorides. J. Org. Chem.1966 31: 838-841.
15. Sacconi L. Acytlhydrazones of o-Oxy- and o-Aminoaldehydes and ketones as tridentate complexing agents. J. Amer. Chem. Soc.1953; 75: 5434-35.

16. Colthup NB, Daly LH, Wiberley SE. Introduction to Infrared and Raman Spectroscopy, 3rd edn., Academic Press, San Diego, 1990.
17. Margaretra A, Mateescu G H. Infrared Spectroscopy: Applications in Organic Chemistry, John Wiley and Sons, Inc., New York.1970.
18. Silverstein RM, Bassler GC, Morrill TC. Spectroscopic Identification of Organic Compounds", now in its 5th edition, 1991, John Wiley & Sons, NY.
19. Dyer J R. Applications of Absorption Spectroscopy of Organic Compounds, Prentice Hall.1974.
20. Cavanagh F. Analytical Microbiology, Academic Press, New York, 1963.