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Synthesis and antifungal activity of 2-(4'substitutedanilinosydnon-3'-yl) pyridines

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ABSTRACT

2-(sydnon-3'-y1) Pyridine[3] was synthesized by heating acetic anhydride with 2-N-Nitroso-N- (pyridin-(2'-yl)] imino acetic acid[2]. The Bromination of compound[3] in the formation of 2-(4' bromosydnon-3'-yl) Pyridine[4]. The final componds 2-[4' substituted anilino sydon-3'- yl) pydines (5a-5e) were obtained by mannich reaction of compounds[4] was substituted aromatic animes. The structure of these compounds have been confirmed by IR, 'H NMR and mass analysis. The newly synthesized compunds have been evaluated for their antifungal activity compound (5a) was found to be equipotent to the standards against C. albicans, C. Parapsilosis 22019, A niger, and A. flavus.

Key words: Pyridine derivatives and antifungal activity.

INTRODUCTION

Among the wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, pyridine have played an important role in the medicinal chemistry. Literature survey reveals that pyridine derivatives have attracted considerable attention as they are endowed with wide specrum of activities like antifungal[1-2], insecticidal[3], antibacterial[4-5], berbicidal[6], antimicrobial[7-8], pesticidal[9-10] anti tubercular[11] etc. Pyridine derivatives have proven to be of great importance in exhibiting and enhancing the biological activities. In contrast to this several scientists have reported potent antifungal activity in various pyridine derivatives.

Chemistry

The synthetic path way of the tital compound is show in scheme. Compound 1 i.e. 2-[pyridin-2(2'-y') aminoaceticacid was prepared by reacting 2-amino pyridine with chloro acetic acid. Compound 1 was reacted with sodium nitrate and hydrogen chloride to yield 2-N-Nitroso-N-[Pyridin-(2'-yl) iminoaceticacid 2. Further more, compund 2 treated with acetic anhydride resulted in the formation of 2-[sydnon-(3'-yl)] pyridine 3. The bromination in the presence of ethanol of compound 3 in the formation of 2-(4' Brono- sydnon-3'-yl) pyridine (4). The final compound 2- (4' substituted onilinosydnon-3'-yl) pyridines (5a-5e) were obtained by mannich reaction of compound (4) was substituted aromatic amines.

EXPERIMENTAL

The melting points were determine in open capillaries with an electrothamal melting point apparatus and are uncorrected. Homogereity of the newly synthesized compounds was checked by thin

layer charomatography. The IR spectra were recoreded on Brucket IFS-66 FTIR instrument. 'H NMR spectra were recoreded in $CDCl_3$ or DMSo –D6 on Jeal GSX-400 FT HMR instrument. Chemcial shifts (g) are in ppm and telranethyl silane (TMS) was used an in internal reference. The elemental analysis (C,H,N) of these newly synthesized compounds was caried out on carloerba01108 elemental analyzer. The purity of the compounds was checked by thin layer chronatography on silia Gel. G. plates of 0.5m thickness and spots were visulized in Iodine vapoulr.

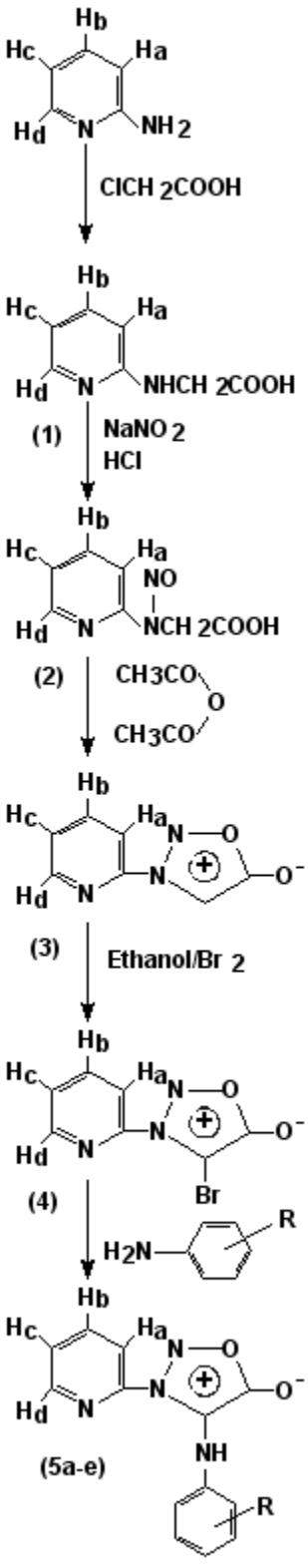
Synthesis of-2-[Pyridin-(2´-yl)]aminoaceticacid (1)

A mixture of 2-amino pyridine (0.01 mol), chloro acetic acid (0.01 mol), and anhydrous K_2CO_3 (5.0 g) in acetone (dry, 80ml) were refluxed for 18-20 hours on a water bath. The solvent i.e acetone was distilled off and the resulting solid mass poured into water, filtered and then separated solid recrystallised from methanol-water to give compound **1**.

M.P. 87°C; Yield 79%; Molecular Formula $C_7H_8N_20_2$ (152).Elemental Analysis. Calcd C, 55.26; H 5.26, N,18.42. found C 55.41, H 5.19, N 18.38.IR (KBr) δ (cm^{-1}): 1555 (C....C of aromatic), 1622 (C=N), 1712 (C=O of COOH), 2850 (CH_2), 3005 (OH of COOH), 3043 (aromatic CH), 3335 (NH).^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 4.60 (d, 2H, CH_2), 6.55 (dd, 1H, H_b), 7.18 (ss, 1H, NH, exchangeable with D_2O), 7.52 (d, 1H, H_c), 7.24 (dd, 1H, H_d), 8.36 (d, 1H, H_a), 9.49 (s, 1H, COOH, exchangeable with D_2O).
MS: $[M]^+$ m/z 152.

Synthesis of-2-N-nitroso-N-[pyridin-(2´-yl)] iminoaceticacid (2)

To a well stirred mixture of compound **1** (0.01 mol) in 40% hydrochloric acid (0.01 mol)at 0-5 °C, a solution of sodium nitrite (0.01 mol)in water (25 ml) was added dropwise during 30 min. The reaction was allowed to stand overnight, filtered, washed thoroughly with ice cold water and dried in air. The solid thus obtained was recrystallised with ethanol-water. M.P. 96°C; Yield 65%; Recrystallisation Solvent Benzene; Molecular Formula $C_7H_7N_3O_3$ Elemental Analysis. Calcd C,



Scheme 1

46.40; H 3.86, N,23.20. found C 46.72, H 3.60, N 23.35.IR (KBr) δ (cm^{-1}): 15480 (C....C of aromatic), 1610 (C=N), 1720 (C=O of COOH), 2830 (CH_2), 3015 (OH of COOH), 3051 (aromatic CH).^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 4.69 (d, 2H, CH_2), 6.62 (dd, 1H, H_b), 7.40 (d, 1H, H_c), 7.35 (dd, 1H, H_d), 8.39 (d, 1H, H_a), 9.58 (s, 1H, COOH, exchangeable with D_2O).
MS: $[M]^+$ m/z 181.

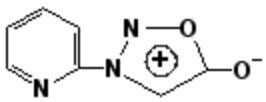
Synthesis of-2-[Sydnon-(3´-yl)]pyridine (3)

Compound **2** was heated with acetic anhydride (1:5 by weight) on a water bath for 3-5 hours.The reaction mixture was then poured in crushed ice and recrystallised to get compound **3**.M.P. 135°C; Yield 65%; Recrystallisation Solvent Ethanol/water; Molecular Formula $C_7H_5N_3O_2$ Elemental Analysis. Calcd C, 51.53; H 3.06, N,25.76. found C 51.70, H 3.35, N 25.90.IR (KBr) ν (cm^{-1}): 849 (N-O of sydnone), 1085 (C-O of sydnone), 1246 (C-N), 1530 (N-N), 1565 (C....C of aromatic), 1628 (C=N), 1745 (C=O of sydnone), 3045 (aromatic C-H). ^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 6.38 (s, 1H, sydnone CH), 7.50-8.30 (m, 4H, Ar-H).
MS: $[M]^+$ m/z 163.

Synthesis of 2-(4´-Bromosydnon-3´-yl)pyridine (4)

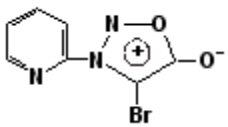
To a suspension of compound **3** (1.0g, 0.005 mol) in ethanol (40 ml), sodium bicarbonate (2.0g, 0.024 mol) was added at room temperature. The cloudy solution thus obtained becomes clear

Table 1 : Physical and analytical data of 2-(sydnon-3'-yl)pyridine (3)



Compd. No.	X	R	M.P. (°C)	Yield (%)	Recrystallisation solven	Molecular formula	Elemental Analysis (%) Calculated/Found C	H	N
3.	-	-	135	65	Ethanol /water	$C_7H_5N_3O_2$	51.53/ 51.70	3.06/ 3.35	25.76/ 25.90

Table 2 : Physical and analytical data of 2-(4'-bromosydnon-3'-yl)pyridine (4)



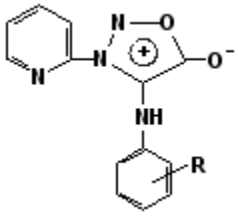
Compd. No.	X	R	M.P. (°C)	Yield (%)	Recrystallisation solven	Molecular formula	Elemental Analysis (%) Calculated/Found C	H	N
4.	-	-	198	62	Acetone	$C_7H_4N_3O_2BR$	34.71/ 34.50	1.65/ 1.94	17.35/ 17.15

on addition of bromine (0.9g, 0..005 mol) in ethanol (25 ml). The reaction mixture was stirred further for 30 min and diluted with water (200 ml). The solid thus obtained was filtered and recrystallised.M.P. 198°C; Yield 52%; Recrystallisation Solvent Acetone Molecular Formula $C_7H_4H_3O_2Br$ Elemental Analysis. Calcd C, 34.71; H 1.65, N,17.35, found C 34.50, H 1.94, N 17.15.IR (KBr) δ (cm^{-1}): 610 (C-Br), 835 (N-O of sydnone), 1090 (C-O of sydnone), 1252 (C-N), 1522 (N-N), 1570 (C....C of aromatic), 1620 (C=N), 1755 (C=O of sydnone), 3030 (aromatic C-H).^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 7.30 (dd, 1H,H_c of pyridine), 7.39 (d, 1H, H_d of pyridine), 7.50 (dd, 1H, H_b of pyridine), 8.30 (d, 1H, H_a of pyridine).
MS: $[M]^+$ m/z 242.

Synthesis of 2-(4´-Substitutedanilinosydnon-3´-yl)pyridines (5)

The solution of compound **4** in methanol (80 ml) was refluxed with ortho-methoxy aniline (0.02 mol) for 7-8 hours. Excess of solvent was distilled off and the reaction mixture thus obtained was cooled, poured into ice cold water, washed with petroleum ether (40°-60°C) and recrystallised furnish the product.2-(4´-p-methoxyanilinosydnon-3´-yl)pyridine (5a) M.P. 212 °C; Yield 58%; Recrystallisation Solvent Ethanol/ water; Molecular Formula $C_{14}H_{12}N_4O_3$ Elemental Analysis. Calcd C, 59.15; H 4.22, N,19.71. found C 46.38, H 4.10, N 19.95.IR (KBr) δ (cm^{-1}): 843 (N-O of sydnone), 1185 (C-O of sydnone), 1260 (C-N), 1530 (N-N), 1580 (C....C of aromatic), 1610 (C=N), 1740 (C=O of sydnone), 3040 (aromatic C-H), 3115 (C-H), 3315 (N-H).^{1}H-NMR ($CDCl_3$+DMSO-d_6) δ (ppm): 3.70 (s, 3H, OCH_3), 5.50 (brs, 1H, NH-Ar, exchangeable with D_2O), 7.45 (dd, 1H, H_c of pyridine), 7.80 (d, 1H, H_d of pyridine), 7.55 (dd, 1H, H_b of pyridine), 7.15-7.80 (m, 4H, Ar-H), 8.35 (d, 1H, H_a of pyridine).
MS: $[M]^+$ m/z 284.

Table 3 : Physical and analytical data of 2-[4'-substitutedanilinosydnon-3'-yl)pyridine (5a-5e)



Compd. No.	X	R	M.P. (°C)	Yield (%)	Recrystallisation solvent	Molecular formula	Elemental analysis (%) calculated/found		
							C	H	N
5a.	-	p-OCH_3	212	58	Ethanol/ water	$C_{14}H_{12}N_4O_3Br$	59.15/ 59.38	4.22- 4.10	19.71/ 19.95
5b.	-	o-OCH_3	222	52	Acetic Acid	$C_{14}H_{12}N_4O_3Br$	46.15/ 46.03	4.22/ 4.08	19.71 19.58
5c.	-	m-Cl	259	60	Ethanol/ water	$C_{13}H_9N_3O_2Cl$	54.07/ 54.33	3.11/ 3.44	19.41/ 19.18
5d.	-	o-Cl	251	59	Ethanol/ water	$C_{13}H_9N_3O_2Cl$	54.07/ 54.26	3.11/ 2.96	19.41/ 19.65
5e.	-	H	254	55	Methanol/ water	$C_{13}H_{10}N_3O_2$	61.41/ 61.18	3.93/ 3.68	22.05/ 22.33

Compounds **5b, 5c, 5d,** and **5e** were prepared from compound 4 following the same procedure as mentioned for compound 5a. Physical and analytical data of compounds 5a-5e is given in Table 3.

Antifungal activity

Compounds 5a-5e of Scheme were evaluated for their antifungal activity. All these compound were tested against Aspergillus flovus, A niger and A. fumigatus, C. albicans, C. albicans, ATCC, C glabrata, C.parapsilosis. Candida spp. and C. Krusei at Nicolas Piramol Ltd. Mumbai.

Poisened food technique

The study were performed by Gehlot and Vohra,[12] to evaluate the antifungal propery of the test compounds and standard drugs i.e. fluconazole and griseofulvin against *Aspergillus flavus*, *A. niger,* and *A. fumigatus*.

10% solution of DMSO in methanol was prepared. 100 mg of test compound as well as the reference drugs i.e fluconazole and griseofulvin were dissolved in sufficient amount of this solution (5 ml). This solution (5 ml) was added to 995 ml Czapex Dox Agar medium so as to obtain 100 mg /L concentration of the compound in the medium. 5ml of 10% DMSO in methanol solution (without any test compound or the standard drug) added to 995ml Czapex Dox Agar medium served as control. The resultanat solutions were thoroughly mixed and approximately 20 ml of the solution was poured into 9cm sterile glass Petridishes and allowed to set. The resulting agar plates were inoculated with 5 mm plugs of fungal mycelia cut from freshly prepared, actively growing cultures. The plates were then incubated at 25 ±°C in the dark for eight days. The diameter of each colony was measured after eight days of incubation. Three replicates were taken for each test compound and for each organism test cultures. The average inhibition due to the given test compound was calculated using the equation:

$$\text{Inhibition \%} = (C-T)\ 100 / C$$

Wherein;

C = Diameter of the fungal colony in mm in the control medium.

T = Diameter of the fungal colony in mm in the test medium containing the given test compound or the reference drug.

Standard agar disc diffusion method

The diffusion method were performed by pai and platt[13]. All the cultures were maintained of Sabouraud Dextrose Agar medium and incubated at 30 °C. In order to prepare homogenous suspension of these fungi for disc assays, they were grown overnight in Sabouraud broth, centrifuged to collect the pellet and resuspended in sterile phosphate buffered saline. The fungal pellet was homogenized in sterile hand held homogenizer. This suspension was then plated on a Sabouraud Dextrose Agar medium using a bacterial spreaded to obtain an even growth. Sterile 6 mm whattmann filter paper disc were impregnated with 100mg/ L of various test compounds and standard drugs. These discs were then placed in the centre of quadrent of Sabauroud Dextrose Agar medium plate. These plates has one control disc impregnated with 10% DMSO in methanol. These plates were incubated at 30°C. Three replicates were used for each test compound as well as for each standard drug used. After 48 hours the plates were removed and radii of inhibition zone were measured and the average calculated.

RESULTS AND DISCUSSION

Compounds 3, 4, and 5a-5e were assayed for antifungal activity against five *Candida* strains i.e *Candida albicans, C. albicans ATCC, C. krusei* G03*, C. glabrata H05, C. parapsilosis 22019* and three *Aspergillus* strains i.e *A. fumigatus, A. niger,* and *A. flavus*. The activity of all these tested compounds was compared with the standard drugs fluconazole and griseofulvin at a concentration of 100 mg/L. (Table 4 and Table 5). All the compounds exhibited antifungal activity and compound **5c** was found to be the most potent compound of this series, possessing better activity as compared to the standard drugs against *Candida albicans, C.parapsilosis 22019*, and *A.niger*. However it showed equipotency with the reference drugs against *C.krusei G03, C.glabrata H05*, and *A.flavus*. Compound **5a** was found to be equipotent to the standards against *C.albicans, C.parapsilosis 22019, A.niger*, and *A.flavus*. (Table 4 and Table 6).

Table 4: Pharmacological data of compounds 2, 3, 4, and 5a-5e

Compounds	Antifungal activity# [diameter of inhibition zone (mm)]				
	Candida albicans	Candida albicans ATCC	Candida krusei GO3	Candida glabrata HO5	Candida parapsiolsis 22019
@ Control	0	0	0	0	0
Fluconazole*	29	25	19	15	20
Griseofulvin*	25	26	18	16	22
3.	12	17	-	-	16
4.	16	19	12	-	17
5a.	24	19	15	-	21
5b.	23	15	13	-	14
5c.	35	21	20	17	27
5d.	22	18	15	-	17
5e.	21	18	10	-	-

Concentration was 100 mg/L.

@ 10% DMSO is methanol.

– : No inhibition zone.

* Standard drugs used for comparison.

Table 5: Pharmacological data of compounds 2, 3, 4, 5a-5e.

Compounds	Antifungal activity# [Inhibition in percentage]		
	Aspergillus fumigatus	*Aspergillus niger*	*Aspergillus flavus*
@ Control	0	0	0
Fluconazole*	-	90	84
Griseofuloin*	80	88	82
2.	-	-	-
3.	-	46	-
4.	-	54	43
5a.	54	88	84
5b.	39	75	46
5c.	68	94	82
5d.	46	78	69
5e.	-	62	-

Concentration was 100 mg/L.

@ 10% DMSO is methanol.

– : No inhibition zone.

* Standard drugs used for comparison.

The characteristic feature of compound **3** is the presence of sydnone nuclei at two position of pyridine ring. It exhibits mild antifungal activity, which shows that presence of sydnone ring in the compound introduces the antifungal activity.

Further it could be noted from the results that presence of bromo substituted sydnone moiety in comound **4** revealed better activity as compared to its parent compound i.e compound **3**. This increased activity of compound **4** may be due to the presence of bromo group at four position of sydnone nuclei in this compound.

Compounds **5a-5e** possessing substituted aniline ring shows moderate to impressive antifungal activity. Out of five aniline derivatives (**5a, 5b, 5c, 5d,** and **5e**), compound **5c** was found to be most active compound of the series. It exhibited most potent activity in comparision to its other derivatives as well as to that of the standard drugs. Compound **5a** also showed satisfactory results. Its activity was however not better than the standards but was found to be equipotent when compared with them against *Candida albicans, C.parapsilosis 22019, Aspergillus niger,* and *A.flavus.*

It is important to note from the biological data that compound **5c** with o-chloroaniline showed remarkable activity, compound **5a** with o-methoxyaniline showed equipotency. Other substituents i.e p-methoxyaniline, p-chloroaniline, and unsubstitutedaniline (compounds **5b, 5d, 5e**) displayed less but still adequate activity.

CONCLUSION

- Presence of sydnone nuclei introduces the antifungal activity in the compounds.
  The presence of bromo group at 4-position of sydnone nuclei enhances the activity as well as widens the spectra of antifungal activity.
  The presence of substituted aniline moiety increases the antifungal activity, producing impressive results.
  Appearance of chloro and methoxy group at ortho or para position produces the compounds with promising antifungal activity. Ortho derivatives however revealed better antifungal activity.

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