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Synthesis and Biological Activity of Phenyl Amino Acetic Acid (2-Oxo-1,2-dihydroindol-3-ylidene)hydrazides

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In present work, some new phenyl amino acetic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazides (**4**) were prepared. Aniline was refluxed with ethyl cholroacetate in dry acetone in presence of anhydrous potassium carbonate to prepare ethylphenylaminoacetate (**1**) which on subsequent reaction with hydrazine hydrate to yield phenyl amino acetic acid hydrazide (**2**). This compound was refluxed with different isatins (**3**) to get the phenyl amino acetic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazides (**4**). The structures of the products were characterized by spectral studies (IR, PMR and mass). All the compounds were evaluated for antibacterial and antifungal activities.

Key Words: Synthesis, Biological activity, Isatin, Phenyl amino acetic acid hydrazide.

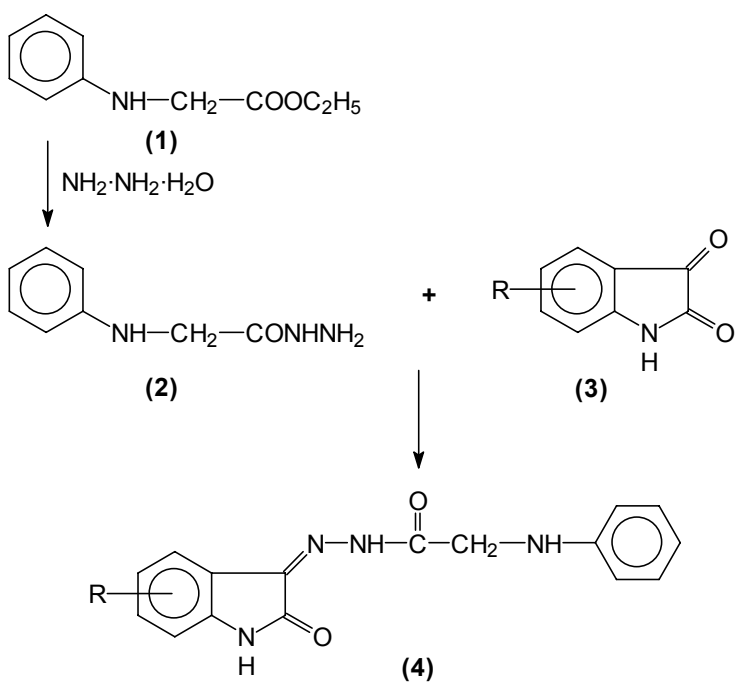
INTRODUCTION

Isatin derivatives are biologically important heterocyclic compounds associated with antibacterial[1,2], antifungal[3], antiviral[4], anticancer[5,6], anti-inflamatory[7-9] and CNS activities[10,11]. Compounds containing hydrazones possess antimicrobial properties[12]. In view of biological significance of isatin derivatives and hydrazone moiety; it is worthwhile to synthesize phenyl amino acetic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazides by molecular conjunction method. The compounds were characterized by spectral data. Aniline reacted with ethylchloroacetate in dry acetone in presence of anhydrous potassium carbonate to prepare ethylphenylamino acetate (**1**). This compound **1** on condensation with hydrazine hydrate in alcohol produced phenylamino acetic acid hydrazide (**2**) in good yield which on refluxing with different isatins (**3**) in alcohol and traces of acetic acid afforded phenyl amino acetic acid (2-oxo-1,2-dihydroindol-3-ylidene)-hydrazides (**4**). These compounds were screened for antibacterial and antifungal activities by cup-plate method[13,14].

EXPERIMENTAL

Melting points were determined in open capillary tubes, using Toshniwal melting point apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer spectrum BX-I series, FT-IR spectrophotometer using KBr discs. PMR spectra were recorded on brucker spectrospin 400 MHz spectrophotometer using TMS as an internal standard.

Synthesis of the title compounds was effected as shown in the **Scheme-I**. The required isatins were prepared by using the method available in literature[15].



Scheme-I

Synthesis of ethyl phenyl amino acetate (1): Ethyl chloroacetate (0.012 mol) was refluxed with aniline (0.01 mol) on water bath in dry acetone (25 mL) and anhydrous potassium carbonate (0.01 mol) for 6 h. The solvent was evaporated and the reaction mixture was poured into crushed ice to get the respective ethyl phenyl amino acetate. The solid thus separated was filtered, dried and recrystallized from petroleum ether. The compound was characterized by the physical constant available in literature, m.p. 55 ºC.

IR spectrum (KBr, ν_{max}, cm^{-1}) of compound recorded absorption bands at 3319 (NH), 2989 (C–H) 1703 (C=O), 1599 (C=C).

Synthesis of phenyl amino acetic acid hydrazide (2): Ethyl phenyl amino acetate (**1**) (0.01 mol) was refluxed on water bath with excess of hydrazine hydrate (0.02 mol) in alcohol (25 mL) for 4 h. The solvent was evaporated, the product thus obtained was washed with cold water, dried and purified by recrystallization with suitable solvent(s). The compound was characterized as a phenylamino acetic acid hydrazide (**2**) by physical and spectral data and m.p. 125-127 ºC.

IR spectrum (cm^{-1}) of the compound recorded its absorptions at 3309 ν(NH_2), 3130 ν(NH), 2985 ν(C–H), 1710 ν(C= O), 1590 ν(C=C). PMR (DMSO-d_6) of the compound exhibited characteristic peaks (δ ppm) at 10.9 (S, 1H, CONH), 9.1 (S, 1H, NH), 6.5-7.1(m, 5H, Ar-H), 5.8 (S, 1H, NH), 4.2 (S, 2H-CH_2).

Synthesis of phenyl amino acetic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazides (4): Each of the isatin (**3**) (0.001 mol) was condensed with phenylamino acetic acid hydrazide (0.001 mol)) in alcohol (25 mL) containing traces of acetic acid on water bath for 3 h to get the respective phenyl amino acetic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazide (**4**). The solvent was evaporated and the reaction mixture was poured into crushed ice. The product thus separated was filtered, dried and recrystallized from alcohol. Similarly, all the compounds were prepared and the purity was checked by TLC. The compounds were characterized by physical and spectral data.

Isatin (**3a**, R=H) and phenylamino acetic acid hydrazide were refluxed in alcohol containing two to three drops of acetic acid to get crystalline yellow compound m.p. 195-200ºC.

IR spectrum (KBr, cm^{-1}) of the compound (**4a**, R=H) showed absorptions at 3390 ν(NH), 2923 ν(C-H), 1694 ν(C=O), 1621 ν(C=O), 1605 ν(C=N), PMR Spectrum (DMSO-d_6) of the compound exhibited its characteristic peaks (δ ppm) at 11.25 (S, 1H, NH lactam), 10.8 (S, 1H, CONH), 6.6-7.6 (m, 9H, Ar-H), 6.2 (S, 1H, NH), 4.2 (S, 2H, CH_2). The physical data of the compounds is presented in Table-1.

Antibacterial and antifungal activity by cup plate method: The antibacterial activity of synthesized compounds was conducted against gram-positive bacteria *viz.*, *Bacillus stereothermophilus* and *Staphylococcus aureus* and gram-negative bacteria *viz.*, *Escherichia coli* and *Kl. aeruginosa* using cup plate method. Ampicillin sodium was employed as standard to compare the results. All those compounds screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were: *Microsporum gypseum* and *Aspergillus niger* using clotrimazole as reference standard. Solutions of the test compounds were prepared by dissolving 10 mg each in 0.1 % NaOH (10 mL).

TABLE-1
PHYSICAL DATA OF THE COMPOUNDS [**4(a-o)**]

Compd.	R	m.p. (ºC)	Yield (%)	m.f. (m.w.)
4a	H	198	95	$C_{16}H_{14}N_4O_2$ (294.31)
4b	5-CH_3	230	95	$C_{17}H_{16}N_4O_2$ (308.13)
4c	7-CH_3	222	80	$C_{17}H_{16}N_4O_2$ (308.13)
4d	5-Cl	220	90	$C_{16}H_{13}N_4O_2Cl$ (328.07)
4e	7-Cl	200	78	$C_{16}H_{14}N_4O_2Cl$ (328.07)
4f	5-NO_2	240	70	$C_{16}H_{13}N_5O_4$ (339.10)
4g	7- NO_2	230	68	$C_{16}H_{13}N_5O_4$ (339.10)
4h	5-Br	200	90	$C_{16}H_{13}N_4O_2Br$ (372.02)
4i	6-Br	260	95	$C_{16}H_{13}N_4O_2Br$ (372.02)
4j	5-COOH	208	50	$C_{17}H_{14}N_4O_4$ (338.10)
4k	5-$COOCH_3$	210	60	$C_{18}H_{16}N_4O_4$ (352.12)
4l	7- $COOCH_3$	190	68	$C_{16}H_{14}N_4O_2$ (352.12)
4m	5-F	230	85	$C_{16}H_{13}N_4O_2F$ (312.10)
4n	5-I	215	45	$C_{16}H_{13}N_4O_2I$ (420.00)
4o	4-Cl, 5-F	215	90	$C_{16}H_{12}N_4O_2ClF$ (346.02)

All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 % sodium hydroxide to observe the solvent effects.

RESULTS AND DISCUSSION

Compounds were evaluated for their antibacterial activity against both gram-positive, gram-negative bacteria and antifungal activity. The results of the evaluation were viewed by taking ampicillin (10 µg/cup), a broad-spectrum antibiotic as the standard drug for antibacterial activity and clotrimazole (10 µg/cup) for antifungal activity.

Table-2 pertaining to the antibacterial activity data of phenyl amino acetic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazides indicates that these compounds showed moderate antibacterial activity. Amongst them, compounds **4h** and **4n** (R = 5-Br and 5-I) were found to be relatively more effective and active against all four strains of bacteria showing a zone of inhibition of 5.44, 1.84, 5.12, 0.96 and 5.68 mm, 3.28, 2.08 and 1.06 mm, respectively. It is also noticed from the data that the compounds with **4f** (5-NO_2) and **4o** (4Cl, 5F), substituents were next in the order of antibacterial activity and very few compounds (5-$COOCH_3$, 7-$COOCH_3$) were active against only one organism. The compounds with (R=H and 5-CH_3) were active against only gram-positive organisms *i.e.*, *B. stereothermophilus* and *S. aureus* whereas compound with R = 7-CH_3, 6-Br and 7-Cl did not show antibacterial activity against all the organisms employed.

The antifungal data of phenyl amino acetic acid (2-oxo-1,2-dihydro-indol-3-ylidene)hydrazides reveals that the compounds **4a** (R=H), 4b (R = 5-CH_3) **4d** (R = 5-Cl), **4e** (R = 7-Cl), **4h** (R = 5-Br) and **4i** (R=6-Br) showed antifungal activity (Table-2). Compound with 5-I and 6-Br substitutions were found to be relatively more effective against two strains of fungi showing average zone of inhibition of 4.3 and 3 mm. It was also noticed from the data that the compounds with 7-CH_3 and 5-$COOCH_3$ substituents were next in the order of antifungal action whereas the compounds with 4-Cl, 5-F, 7-$COOCH_3$, 5-COOH and 7-NO_2) did not show any activity against any strain of the fungi.

TABLE-2
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES
Zone of inhibition of the compounds **4(a-o)** (mm)

Compd.	R	KA	EC	BS	SA	MG	AN
4a	H	–	–	8.86	0.88	3.2	0.8
4b	5-CH_3	–	–	2.32	0.96	1.5	1.2
4c	7-CH_3	–	–	–	–	1.6	–
4d	5-Cl	–	1.6	–	–	1.2	1.1
4e	7-Cl	–	–	–	–	1.0	1.4
4f	5-NO_2	2.08	1.6	2.64	1.84	–	1.0
4g	7- NO_2	1.68	-	3.0	-	–	–
4h	5-Br	5.44	1.84	5.12	0.95	2.1	3.5
4i	6-Br	–	–	–	–	3.4	2.6
4j	5-COOH	–	-	2.52	2.16	--	–
4k	5-$COOCH_3$	–	1.84	–	–	0.3	–
4l	7- $COOCH_3$	–	1.72	–	–	–	–
4m	5-F	–	3.2	–	2.08	2.1	1.1
4n	5-I	5.68	3.28	2.08	1.36	4.7	3.9
4o	4-Cl, 5-F	1.68	–	3.28	2.48	–	–
Ampicillin (10 µg/cup)		10	10	–	–	–	–
Clotrimazole (10 µg/cup)		–	–	–	–	8	7

KA = *Kl. Aeruginosa*, EC = *E. coli*, BS = *B. steriothermophilus*,
SA = *S. aureus*, MG = *M. gypseum*, AN = *A. niger*
Concentration of the test compound: 10 µg/cup

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REFERENCES

1. R.S. Varma and R.K. Pandeya, *Indian J. Pharm. Sci.*, **46**, 132 (1982).
2. K. Anil, M.M. Sengupta and A.A. Gupta, *J. Indian Chem. Soc.*, **61**, 643 (1984).
3. F.H. Havaldar and S.K.J. Mishra, *Asian J. Chem.*, **16**, 1971 (2004).
4. A. Ferranti, L. Garuti, G. Giovanninetti and E. Katz, *Arch. Pharm. (Weinheim)*, **318**, 415 (1985).
5. G.R. Pettit and J.A. Settepin, *J. Org. Chem.*, **27**, 1714 (1962).
6. R.C. Elderfield and J.R. Wood, *J. Org. Chem.*, **27**, 2463 (1962).
7. J. Debat, Ger. Offen., 1,935,697 (1970); *Chem. Abstr.*, **72**, 125053 (1970).
8. N. Hirose, S. Sohda, S. Kuriama and S. Toyoshima, *Chem. Pharm. Bull. (Tokyo)*, **21**, 960 (1973).
9. S. Sanjay, V.K. Saxena and S.R. Chowdary, *Indian Drugs*, **27**, (1990).
10. S. Dilber, A. Gelineo, M. Bogava, L. Arsenijevic, M. Saban and J. Jelaca, *Pharmazie*, **44**, 649 (1989).
11. S.N. Pandeya, M. Verma, K. Singh and J.P. Stables, *Acta Pharm.*, **54**, 49 (2004).
12. R.G. Johnston and W. Kidd, *J. Chem. Soc.*, 4734 (1964).
13. Indian Pharmacopoeia, Microbiological assay and test, Vol. 2, A 100-107 (1996).
14. British Pharmacopoeia, Pharmaceutical Press, London, p. 796 (1953).
15. C.S. Marvel and G.S. Heirs, Organic Synthesis, Collective Vol. 1, p. 327 (1941).

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