Synthesis, Characterization and Substituent Effect on Biological Properties of Schiff bases Derived from Anthranilic Acid

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ABSTRACT
The effect of substituents on biological activity of Schiff bases was investigated using Schiff bases derived from the condensation of oxindol with, 4-methyl anthranilic acid, 4-methoxy anthranilic acid, 4-nitro anthranilic acid, 4-chloro anthranilic acid and 4-hydroxy anthranilic acid. The synthesized compounds are characterized by elemental analysis, IR and ^{1}HNMR. The compounds were screened against important bacteria namely *Staphylococcus aureus*, *Escherichia coli* and *Bacillus Subtilis* bacterial strains and for in vitro antifungal activity against *Candida albicans* and *Aspergillus niger* using the agar cup plate method and radial growth method respectively and results show that 4-chloro anthranilic acid Schiff base exhibited highest activity.

Keywords
Oxindol, Schiff base, Substituent effect, biological activity, ^{1}HNMR

1. INTRODUCTION
Over the past decade, the synthesis of privileged classes of heterocyclic molecules has become one of the main areas of interest in synthetic chemistry[1,2]. Schiff bases are important class of ligands due to their synthetic flexibility, their selectivity and sensitivity towards the central metal atom, structural similarities with natural biological substances and also due to presence of imine group (-N=C-) which imports in elucidating the mechanism of transformation and racemization reaction in biological system[3]. Literature survey shows that many Schiff bases exhibit biological activities[4-7] such as antifungal, antibacterial, antitumor, anti-inflammatory, and antipyretic, among others, some of them have been used as complexing agent[8-9] and powerful corrosion inhibitors[10]. They synthesized from various compounds[11-13].

Since the electron donating and accepting properties of the ligand and presence of structural functional groups affects the nature of metal complex obtained, knowledge of ligand properties can afford synthesis of metal complexes with tuneable properties. The mode of interaction and inhibition effectiveness of Schiff bases with bacteria and fungi is expected to depend on the molecular structure of the compounds.

This paper presents a series of new Schiff bases with a potential biological activity resulted from the condensation of aromatic amino acids and oxindol. Oxindole is the core structure in a variety of natural products and drugs. These compounds could also act as valuable ligands.

2. EXPERIMENTAL
2.1 Material
All the chemicals are of reagent grade(AR). Solvents were dried and distilled before use according to standard procedures[14].

2.2 Physical Measurements
The melting point of all complexes was determined by open capillary method. Elemental analysis was obtained from standard method[14]. The molecular weight determinations were carried out in a *'Gallenkamp semi-micro Ebulliometer'* using dioxane as the solvent. The electronic absorption spectra of the ligand and complexes were recorded on VEB Zeiss Jena, VSU spectrometer in the range 200–1000 nm in DMF solution (10^{-3} M). IR spectra of the synthesized ligands and its complexes were recorded as KBr pellets on Perkins-Elmer spectrometer. ^{1}H NMR spectra were recorded on a Hitachi NMR- spectrometer in DMSO using TMS as an internal standard.

2.3 Synthesis of the Schiff Base Ligand
The ligands were synthesised by refluxing for 4 hr, 1:1 stoichiometric proportions of oxindole and 4-substituted anthranilic acids for in alcoholic medium in presence of acetic acid and sodium acetate/piperdin as condensing agents. The coloured precipitates formed were filtered, recrystallised from ethanol, dried and preserved in a vacuum desiccators. The general synthetic route of new series of Schiff base compounds are depicted in **scheme-1**. Quantitative yields of ligands were obtained. The authenticity and purity of the compounds was established by elemental analysis, molecular weight, ^{1}HNMR and IR spectra.

2.4 Biological Activities
The *in vitro* biological screening effects of the investigated compounds were tested against three bacterial strains namely *E. coli*, *B. Subtilis* and *S. aureus* and two fungal strains namely *A. niger* and *C. albicans* by the agar cup plate method[15-16] using nutrient agar medium for antibacterial studies and radial growth method[17] potato dextrose agar medium for antifungal studies[18-19].

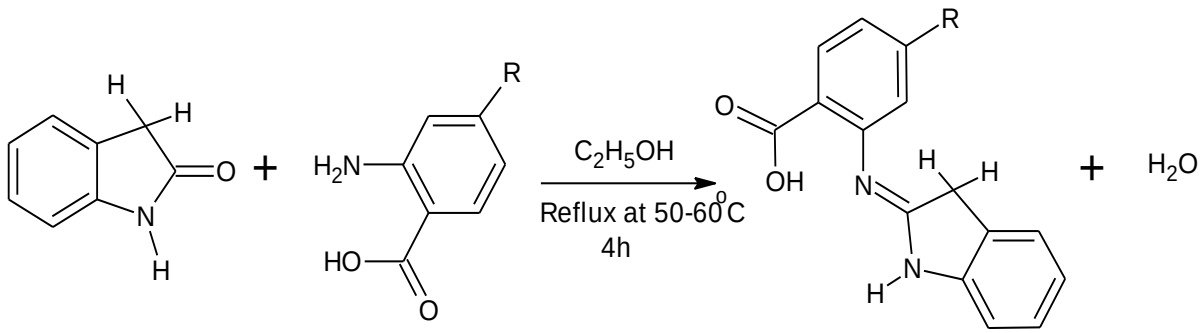
The bacteria and fungi were sub-cultured in the agar and potato dextrose agar medium and were incubated for 24 h for bacteria and 96 h for fungi at 37±2ºC. Standard antibacterial drug (streptomycin) and antifungal drug (fluconazole) were used for comparison. The cup having a diameter of 10 mm fill test solutions and were placed on an appropriate medium

previously seeded with organisms in petri plates and stored in an incubator at the above mentioned period of time. The inhibition zone around each disc was measured and the results have been recorded in the form of inhibition zones (diameter, mm) showed in Table 1. In order to clarify any effect of DMF on the biological screening, separate studies were carried out with solutions alone of DMF and they showed no activity against any microbial strains. The stock solution (1 mg mL^{-1}) of the test compounds was prepared in DMF. Each test was performed in triplicate in individual experiments and the average is reported.

3. RESULTS AND DISCUSSION

3.1 Physico-Chemical Data

The Schiff bases (H_2L_1- H_2L_5) were obtained in moderate yields by condensation of oxindol and the appropriate amino acids namely 4-methyl anthranilic acid**(H_2L_1),** 5-methoxy anthranilic acid**(H_2L_2)**, 4-nitro anthranilic acid**(H_2L_3)**, 4-chloro anthranilic acid**(H_2L_4)** and 4-hydroxy anthranilic acid**(H_2L_5)** under reflux condition (Scheme 1). The physico-chemical data of the Schiff bases are summarized in table-1. The compounds are air stable with sharp melting points indicating the purity of the compounds and microanalysis of the compounds are in agreement with the suggested composition.



R	$-CH_3$	$-OCH_3$	$-NO_2$	-Cl	-OH
Ligand	H_2L_1	H_2L_2	H_2L_3	H_2L_4	H_2L_5

Scheme 1: Synthetic Route of Schiff bases (H_2L_1- H_2L_5)

Table 1: Characterisation of Ligands by Physico-Chemical Parameters

Ligand	Yield %	Colour	MP°C	Mole. Formula	Elemental Analysis % Found (Calc)				Molecular Weight Found (Calc)
					C	H	N	Cl	
o-(N-α-oxindolimino) 4-methylbenzoic acid (H_2L_1)	63	Brown	85	$C_{16}H_{14}O_2N_2$	72.45 (71.18)	5.28 (5.26)	10.56 (10.52)	------	265 (266)
o-(N-α-oxindolimino) 4-methoxybenzoic acid (H_2L_2)	60	Dark Yellow	91	$C_{16}H_{14}O_3N_2$	68.32 (68.08)	4.98 (4.96)	9.96 (9.92)	------	281 (282)
o-(N-α-oxindolimino) 4-nitrobenzoic acid (H_2L_3)	65	Light Yellow	95	$C_{15}H_{11}O_4N_3$	61.02 (60.60)	3.73 (3.70)	14.24 (14.14)	------	295 (297)
o-(N-α-oxindolimino) 4-chlorobenzoic acid (H_2L_4)	62	Yellow	79	$C_{15}H_{11}O_2N_2Cl$	63.01 (62.93)	3.88 (3.85)	9.85 (9.79)	12.32 (12.24)	284 (286)
o-(N-α-oxindolimino) 4-hydroxybenzoic acid (H_2L_5)	65	yellow	81	$C_{15}H_{12}O_3N_2$	67.67 (67.16)	4.53 (4.47)	10.52 (10.47)	------	266 (268)

Table 2: Characterisation of Ligands by Major Peaks in IR Region (4000-400cm^{-1})

Ligands	ν_{-COOH}	$\nu_{>NH}$	$\nu_{>C=N}$	$\nu_{>C=O(S)}$	ν_{-OH} (Phenolic)	$\nu_{-N=O}$ (Nitro)	ν_{-OCH_3} (Methoxy)	ν_{-Cl} (Chloro)
H_2L_1	3012	3310	1620	1692				
H_2L_2	3014	3310	1625	1693			1076	
H_2L_3	3018	3315	1615	1698		1560		
H_2L_4	3015	3312	1610	1704				1078
H_2L_5	3025	3317	1615	1707	3450			

3.2 IR Spectra

Table 2 summarizes the IR spectra of the compounds. The absence of the carbonyl stretch for the oxindol group and presence of an intense band in the region 1610-1625 cm^{-1} attributed to azomethine group (C=N) stretching vibration confirms the formation of the Schiff base. In addition, a band is observed in the range 1692 -1707 cm^{-1} due to (C-O) stretching frequency of the carboxylic group.

3.3 1HNMR

The formation of the compounds was further confirmed by ^{1}HNMR. The resonance of protons has been assigned on the basis of their integration and multiplicity pattern. The signal due to >N-H proton,-COOH proton and -CH_2- proton of the ligands H_2L_1 to H_2L_5 appeared at δ 7.53 -7.84 ppm, δ 12.18-11.89 ppm and δ 1.19-1.22ppm respectively. The multiplets within the δ 7.80-8.2ppm range are assigned to the aromatic protons of both rings. The signals appeared at δ 1.53 (s, CH_3, 3H, H_2L_1), 3.7 (s, CH_3, 3H, CH_3O-, H_2L_2) and 10.8 (s, OH, 1H, H_2L_5), are due to methyl, methoxy and hydroxyl proton respectively.

3.4 UV-Visible Spectra

The electronic absorption spectra of the compounds (H_2L_1 - H_2L_5) were studied in DMF. The electronic spectrum of free Schiff base shows two bands in the region 252-275 and 325-345 nm suggesting the presence of π-π^* transition[20], the first band is due to benzene ring π-π^* transition and second due to n- π^* transition[21] of non bonding electron present on the nitrogen of the imino group. The absorption maximum is shifted to lower wavelength in H_2L_3 which have electron withdrawing substituents and a hypochromic shift in all ligands except H_2L_3 containing an electron releasing substituent.

3.5 Biological Activity

The new compounds H_2L_1-H_2L_5 were assayed *in vitro* to assess their ability to inhibit the growth of selected species of bacteria and fungi using a concentration of 1 mg mL^{-1} of each compound. The diameter (mm) of growth inhibition zones was measured and results are summarized in table 3.

The morphology of the cell wall is a key factor that influences the activity of antibacterial agents. The cell wall of the bacteria is composed of peptidoglycan which is thicker in the Gram positive bacteria and this usually poses a barrier to the degree of diffusion of antibacterial agents into the enzyme[22].On the basis of observed zones of inhibition, it was found that, in general, ligand H_2L_4 was found to be more active against all the selected microbial strains because of the presence of chloro group in the anthranilic group which itself is active against microbes. Antibacterial activity of these compounds shows following order

$$H_2L_4 > H_2L_2 > H_2L_3 > H_2L_5 > H_2L_1$$

When we increase concentration, area of inhibited growth also increased.

4. CONCLUSION

In conclusion, the synthesized new compounds o-(N-α - oxindolimino)4-R-benzoic acid are characterized by spectral data and subjected them for biological assay. All the compounds are potential antibacterial and antifungal agents. Compounds H_2L_4 and H_2L_2 are very good antimicrobial due to the presence of chloro and methoxy group in them.

Table3: Antimicrobial activity data of synthesized compounds (H_2L_1-H_2L_5)

S. No	Ligands	Diameter of Inhibition Zones(mm)				
		E. coli	*B. Subtilis*	*S.aureus*	*A. niger*	*C. albicans*
1	H_2L_1	10	11	10	11	13
2	H_2L_2	13	13	11	14	16
3	H_2L_3	12	12	12	13	15
4	H_2L_4	14	14	13	16	17
5	H_2L_5	11	12	11	12	14

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