RESEARCH ARTICLE ISSN: 2349-2678

Contents lists available at www.ijpba.in

International Journal of Pharmaceutical and Biological Science Archive

Volume 4 Issue 5; September-October; 2016; Page No.12-23

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL SUBSTITUTED-1-(4-SUBSTITUTED BENZYL)-1H-INDOLO (2, 3-B) QUINOXALINE N-BENZYL INDOLE-2,3-DIONE MOIETIES

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ABSTRACT

Indoles are one of the most important alkaloids molecules found extensively in biological systems, which play vital role in many of the biochemical process. Various novel Schiff bases derivatives were synthesized by a chain of reactions. Substituted isonitroso acetanilide were synthesized from substituted aniline and then further substituted-indole-2,3 dione (isatin) subsequently synthesized from substituted isonitroso acetanilide and finally yield different substituted-1-(4-substituted benzyl)-1H-indolo(2,3-b) quinoxaline N-benzyl indole-2,3-dione. All the synthesized compounds were identified by various methods and finally characterized by spectral analysis (IR, MS and NMR). Elemental analysis was also performed. Anti microbial activity of different substituted-1-(4-substituted benzyl)-1H-indolo(2,3-b) quinoxaline N-benzyl indole-2,3-dione was determined. Anti microbial activity was done by turbidity and disc diffusion method. All the compounds were synthesized with good yield. Among the new compounds QX1, QX4 & QX5 are found to most potent anti microbial activity. The results obtained defend the usage of these compounds from their promising antibacterial activity.

Keywords: Isatin, Schiff bases, Quinoxaline N-benzyl indole-2,3-dione, Indole, anti bacterial and anti fungal activity.

INTRODUCTION:

Indole ring constitutes an important basic Skelton and development of the drug [1&2]. The classical indole drugs are indomethacin and indoxole. Indole derivetives found to posses high which includes, antibacterial, analgesic, antipyretic, antifungal, antiinflamatory, anthelmintic, cardiovascu-lar, anticonvalsant and selective COX-2 inhibitary activities [3&4].

R. S. Gani, S. R. Pujar and G.S. Gadaginamath [5] reported the synthesis and antimicrobial activity of some new oxadiazolyl methoxyindole derivatives. The compounds synthesized were screened for their antibacterial and antifungal activities. S. Dundappa Donawade, A V Raghu and S. Guru Gadaginamath [6] studied the synthesis and antimicrobial activity of novel linearly fused 5-substituted-7-acetyl-2,6-dimethyloxazolo[4,5-f] indoles. They were characterized on the basis of their spectral and analytical data. The compounds were further screened for antibacterial and antifungal activites. G. Manjunath Bhovi and S. Guru Gadaginamath [7] studied the synthesis and characterization of some new 1-[2-phenylthioethyl] 1-[2-phenoxyethyl]-3-ethoxycarbonyl-5-methoxy-2-methylindoles/benz[g] indoles. These synthesized indole derivatives were screened for their antimicrobial activity. G. S. Gadaginamath, *et al.* [8] reported the synthesis and antimicrobial activity of some new 3-acetyl-5-(3-methyl-5-hydroxypyrazol-4-yl) oxy-2-methylindoles. Pandeya *et al.* [9] have synthesized the Schiff and Mannich bases of isatin and its derivatives with pyrimidine and reported their antimicrobial activity. Patel *et al.* [10] have synthesized some of the novel isatin derivatives (figure 1) and evaluated for the antimicrobial activity.

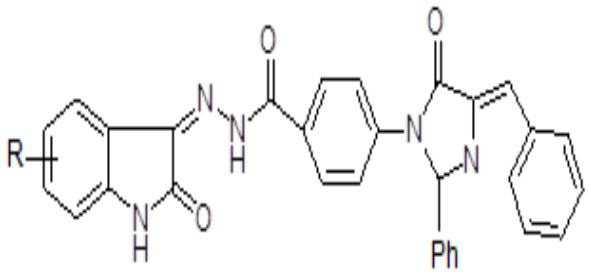

Figure 1: Novel isatin derivatives

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Dorneanu *et al.*, [11] synthesized 6-hydrazones obtained by treating 5-methyl-isatin or 1-morphilino methyl-5-methyl-isatin with 3-(R-phenyl)-6-hydrazino-pyridazine (R= OCH_3,Cl,Br) and two complex combination with copper, derived from 3-(P-anisyl-pyridazinyl) -hydrazone- 5-methyl-indoline-2-one 3-(P-anisyl-pyridazinyl)- hydrazono-5 methyl -indoline -2- one, has a considerable anti-inflammatory activity, giving a inflammation inhibition of 39.6%. The entire synthesized compounds have a moderate antimicrobial activity against Candida albicans.

Thus, in persistence of our continuing program of research on the synthesis of some biologically active compounds, it was thought to be motivating to synthesize compounds containing the features namely, having indole moiety incorporated with different heterocyclic rings and evaluated their antimicrobial activity. In the view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents. The novel derivatives of different substituted-1-(4-substituted benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione were characterized by spectral data and elemental analysis and these compounds were used for their Antimicrobial evaluation screening.

MATERIALS & METHOD

All the chemicals and solvents used in the synthesis of Schiff bases were purchased as LR grade from S. D. Fine Chem. Ltd., Mumbai and Ranbaxy Fine Chemicals Ltd, New Delhi, India and were used directly without any further purification. Melting points were determined by open capillary method with Cambell Electronics, Bombay, India instrument. UV-Visible Spectrometer was UV-1800, Shimadzu, Tokyo, Japan. Infrared spectra (vmax in cm^{-1}) of synthesized compounds were recorded on Prestige-21, Shimadzu, Tokyo, Japan in the range of 400-4000 cm^{-1}. Mass spectra were recorded on JEOL SX 102/ DA-600 instrument. 1HNMR spectra (ppm, δ) were recorded on Bruker ultraspec 500 HZ/AMX 400MHZ/300MHZ spectrometer. CHN elemental analyzer was Perkin-Elmer CHN elemental analyzer.

METHODS:

SYNTHESIS OF SUBSTITUTED-INDOLE-2, 3 DIONE (ISATIN) AND FINAL PRODUCTS:

SCHEME

A. Synthesis of substituted isonitroso acetanilide from substituted aniline

B. Synthesis of substituted-indole-2,3 dione (isatin) from substituted isonitroso acetanilide

C. Synthesis of substituted-1-(4-substituted benzyl) indole-2,3-dione.

D. Substituted-1-(4-substituted benzyl)-1H-indolo(2,3-b) quinoxaline N-benzyl indole-2,3-dione

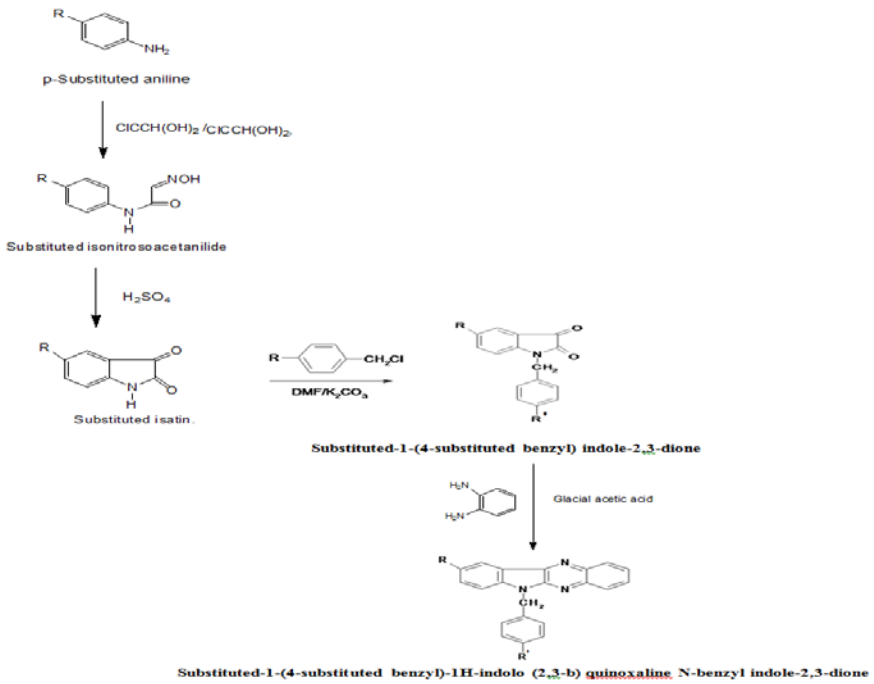


Figure 2: Scheme for synthesis of Substituted-1-(4-substituted benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione from substituted aniline

Where R and R′ are:

Compound	R	R′
QX1	Br	F
QX2	CH_3	F
QX3	CH_3	CH_3
QX4	Cl	CH_3
QX5	Br	CH_3

The experiment work comprises of [3-10]:

(A) Preparation of substituted isonitroso acetanilide from p-substituted aniline:-

100 ml round bottom flask was taken and 3.6 gm (0.05M) of chloral hydrate and 48 ml of purified water was taken in it. Then 44 gm of crystallized anhydrous sodium sulfate was added in it and a solution of substituted aniline (0.05 M) in 12 ml of water with 1.7 ml (0.052M) of concentrated hydrochloric acid was added to dissolve the amine, and finally, a solution of 4.5gm (0.158M) of hydroxylamine hydrochloride in 20 ml of water. The flask was heated over a heating mantle, so that vigorous boiling begins in about 40-45 minutes. After 1-2 minutes of vigorous boiling the reaction completes and during the heating period, some crystal of substituted isonitroso acetanilide separates. The final solution was cooled down under the running water and crystallized material was filtered on suction, and air dried.

(B) Preparation of substituted isatin from substituted isonitroso acetanilide:-

32.5 ml of concentrated sulfuric acid was warmed upto 50°C in a 100 ml round bottom flask with continuous stirring, and 7.5 gram of (0.046 M) of dry substituted isonitroso acetanilide was added to such a rate that to keep the temperature 60-70 but not higher. External cooling was applied at this stage to carry out the reactions more rapidly. After the addition of the substituted isonitroso acetanilide compound was finished, the solution was heated to 80°C and kept at this temperature for about 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured to 10-12 times its volume of cracked ice. After standing for about one and half hour the substituted isatin was filtered with suction, washed several times to cold water to remove the sulfuric acid, and then dried in the air. For purification the dried substituted isatin was dissolved in the 50 ml of hot water and suspension was made, to this hot reaction mixture added the solution of sodium hydroxide (5gm in 10 ml) till the complete dissolution of the substituted isatin. The resulting clear solution was neutralized slowly with dilute hydrochloric acid. Filtered the solution and made the solution acidic with the dilute hydrochloric acid. Cooled the solution and the crystals of different substituted isatins were separated. Filtered the product and dried in oven. The yield values and melting points were recorded of different substituted isatins like unsubstituted Isatin, 5-Chloro isatin, 5-Bromo isatin, 5-Methyl isatin and 5-Nitro isatin, and all the values were compared with reported values.

(C) Method of preparation of substituted N-benzyl indole-2,3-dione from substituted isatin.

In the round bottom flask take indole-2,3-dione (isatin) 0.00337 M and equimolar quantity of benzyl chloride mixed with 20 ml of dimethyl formamide (DMF) and to this mixture added 2 gm of potassium carbonate. After gentle mixing of this reaction mixture, reflux for 2 hour, cooled and poured to 100 ml of ice water cold water. The resultant precipitate collected washed with water and dried and recrystallised from ethanol-water mixture. Dried and checked the melting point.

(D) Method for preparation of substituted 1-benzyl-1H-indolo (2,3-b) quinoxaline FROM substituted N-benzyl indole-2,3-dione.

To the orange colored 1- (4 - substituted benzyl) - 1,3 - di hydro – indole - 2, 3-dione (1gm) equimolar quantity of orthophenylenediamine and 0.50 ml of glacial acetic acid was added and refluxed in 100ml of ethanol for two hours on water bath. The initial Colored solution slowly changes in to some fluffy solid crystals in the end of the reaction, which was verified by TLC on silica plates. Excess ethanol was removed and after drying, the compound purified by ethanol.

IDENTIFICATION AND CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS:

The identification and characterization [8-20] of the compound were carried out by the following procedure to establish the structure and chemical nature of recently synthesized compounds like physical appearance, melting points & boiling points, thin layer chromatography, ultraviolet spectrum, FTIR (FT-Infra red spectroscopy), NMR (1H NMR, 13C NMR), FAB – MS and elemental CHN analysis.

BIOLOGICAL & PHARMACOLOGICAL EVALUATION OF FINAL PRODUCTS

Microbiological Evaluation

All synthesized final products were screened for *in vitro* antibacterial activity [4-10, 14-16] against four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenes, Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282).

All those compounds screened for antibacterial activity were also tested for their antifungal activity by using Agar diffusion cup plate method. The fungi employed for screening were: Aspergillus Niger and Candida albicans. All compounds subjected for antimicrobial activity was performed by two different concentrations and standard drugs like Ciprofloxacin and Fluconazole were used for comparison purpose respectively.

Preparation of Culture Media

Nutrient broth was used as growth medium for bacteria and Saubouraud dextrose broth for fungi. Nutrient broth was prepared by dissolving 12gm of dehydrated powder (HI-media) in 100ml of purified water. Saubouraud dextrose broth was prepared by dissolving 4gm of dextrose and 1gm of peptone in 100ml of distilled water. The media were sterilized by autoclaving at 15lbs pressure for 20 minutes.

Preparation of Stock Culture

Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100ml of sterile broth and incubated for 24 hours at 37^0C for bacteria and 24^0C for fungi.

Standardization of Stock Culture

Stock cultures were placed in the incubator (37^0C for bacteria and 24^0C for fungi) and shaken well. 1ml of stock cultures was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed with using a cyclomixer and serially diluted from 10-1 to 10-10. From each dilution, 0.2ml was taken and spread on sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi, which were incubated for 18 hours. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum dilute tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15 × 108 cfu/ml.

Preparation of Working Stock Culture

Stock culture (0.1ml) was diluted with nutrient broth (100ml) and Sabouraud dextrose broth (100ml) respectively to obtain 105 cfu/ml. This was then used for further *in vitro* screening.

Preparation of Drug Dilutions

Solutions of the title compounds in DMSO (1mg/ml) were prepared and used for screening their antimicrobial activity.

Preparation of discs:

Discs of 5-6 mm in diameter were punched from No. 1 Whattmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C for 60 minutes. Standard and test solutions were added separately to these discs which were air dried later on.

Diffusion Test

A filter-paper disk impregnated with the compound to be tested was placed on the surface of the agar carefully by using sterilized forceps. The compound diffused from the filter paper into the agar. The larger the clear area around the filter disk, the more effective was regarded the compound.

These petridishes were kept up to one hour for diffusion at room temperature and then for incubation at 37°C for 24 hours in an incubator. The

zones of inhibition after 24 hours were measured in millimeters. The size of the zone of inhibition was measured as a determination of compound's effectiveness.

MIC:

Antimicrobial Screening:

Synthesized products were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique. Test was carried out on four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenes, Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282).

Determination of MIC:

The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in triplicate. To the first assay tube, 1.8ml of seeded broth and 0.2ml of title compound (1mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37^0C and 24^0C respectively for 24 hours for bacteria and fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC).

RESULTS & DISCUSSION

Starting materials was identified on the basis of physical characteristics like physical appearance, melting point, boiling point, R_f values, UV spectrums. Physical appearance, melting points, boiling points of all the starting materials e.g., aniline, 4-methyl aniline, 4-chloro aniline and 4-bromo aniline were matched and complied with earlier reported parameters. TLC study was carried out in toluene: methanol solvent system in a ratio of 95:05v/v and R_f values of all the starting materials were calculated and R_f values were fully complied with earlier reported values it was in a range between 0.22±0.06-0.29±0.12. UV spectrums of all the starting materials were fully complied with earlier reported values.

Intermediate and final synthesized compound were characterized on the basis of various parameters like physical appearance, melting points, Thin Layer Chromatography, ultraviolet spectrum, FTIR (FT-Infra red spectroscopy), NMR (1H NMR, 13C NMR), FAB – MS and elemental CHN analysis.

CHARACTERIZATION OF THE SYNTHESIZED COMPOUNDS:

Physical characteristics of Bromo-1-(4-fluro methyl benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione (QX1): was yellowish solids; **Yield:** 83±2.06%; **Mol. Formula:** $C_{21}H_{13}BrFN_3$; **Mol. Wt:** 406.13; **Melting Point:** 213-215°C, **Rf values:** 0.32±0.02, **Maximum wave length (λ_{max})** 284nm; **FTIR Spectra (KBr, cm^{-1}):** 3065-CH stretching (aromatic); 2917-CH stretching (aliphatic); 2848-CH stretching (aliphatic) 1604-C-N stretching; **1H-NMR (DMSO, δppm)–** 8.32-6.95-11H, Ar-H; 5.662H, N-CH_2.

Physical characteristics of Methyl-1-(4-fluro benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione (QX2) was yellowish solid; **Yield:** 79±3.04%; **Mol. Formula:** $C_{22}H_{16}FN_3$; **Mol.Wt:** 341.25; **Melting Point:** 224-226°C, **Rf values-** 0.41±0.06, **Maximum wave length (λ_{max})** in nm was 243; **FTIR Spectra (KBr, cm^{-1}):** 3056- CH stretching (aromatic); 2917-CH stretching (aliphatic); 2848-CH stretching (aliphatic); 1604-C-N stretching; **1H-NMR (DMSO, δppm)–** 8.32-6.9811H, Ar-H; 5.66-2H, N-CH_2; 2.54-3H, CH_3.

Physical characteristics of methyl-1-(4-methyl benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione (QX3) was yellowish solid, **Yield:** 78±2.46%; **Mol. Formula:** $C_{23}H_{19}N_3$, **Mol.Wt:** 337.41; **Melting Point:** 221-223°C, **Rf values-** 0.60±0.14, **Maximum wave length (λ_{max})** in nm was 242; **FTIR Spectra (KBr, cm^{-1}):** 3055-CH stretching (aromatic); 2922-CH stretching (aliphatic); 2852-CH stretching (aliphatic); 1617-C-N stretching; **1H-NMR (DMSO, δppm)–** 8.39-7.15-11H, Ar-H, 5.72-2H, N-CH_2, 2.59-3H, CH_3, 2.34- 3H, CH_3; **^{13}C NMR-**145.99-21.16; MS Spectra-337.42; CHN analysis found (reported) - **C%** 81.29 (81.88), **H%** 4.46 (5.69), **N%**12.25 (12.43).

Physical characteristics of chloro-1-(4-methyl benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione (QX4) was brownish solid, **Yield:** 80±3.94%; **Mol. Formula:** $C_{22}H_{16}ClN_3$, **Mol. Wt:** 357.82; **Melting Point:** 193-196°C, **Rf values-** 0.58±0.12, **Maximum wave length (λ_{max})** in nm was 284; **FTIR Spectra (KBr, cm^{-1}):** 3056-CH stretching

(aromatic); 2916-CH stretching (aliphatic); 2844-CH stretching (aliphatic); 1614 -C-N stretching; **1H-NMR (DMSO, δppm)–** 8.52-7.1611H, Ar-H, 5.71-2H, N-CH_2, 2.36- 3H, CH_3; **^{13}C NMR-**145.84-21.16; **MS Spectra-**357.83; CHN analysis found (reported) - **C%** 73.59 (73.84), **H%** 3.23 (4.51), **N%** 67.01 (11.75).

Physical characteristics of Bromo-1-(4-methyl benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione (QX5) was brownish solid, **Yield:** 80±4.14%; **Mol. Formula:** $C_{22}H_{16}N_3Br$, **Mol.Wt:** 402.26; **Melting Point:** 192 -194°C, **Rf values-** 0.62±0.16, **Maximum wave length (λ_{max})** in nm was 284; **FTIR Spectra (KBr, cm^{-1}):** 3054-CH stretching (aromatic); 2919-CH stretching (aliphatic); 2847-CH stretching (aliphatic); 1607-C-N stretching; **1H-NMR (DMSO, δppm)–** 8.65-7.12-15H, Ar H, 5.67-2H, N-CH_2, 2.28-3H, CH_3; **^{13}C NMR-**145.68-21.16; MS Spectra-402.28.

BIOLOGICAL & PHARMACOLOGICAL EVALUATION OF FINAL PRODUCTS

Microbiological Evaluation-

Antibacterial activity

Table 1: Antibacterial Activity: Zone of inhibition of the synthesized compounds

Compound	Antibacterial Activity: Zone of inhibition (mm)							
	Gm +ve				Gm -ve			
Bacterial strain	Staphylococcus aureus		Staphylococcus pyogenes		Escherichia coli		Pseudomonas aeruginosa	
Concentration (µg/ml)	100		100		100		100	
IZ and AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
QX1	24±2	0.89	22±2	0.85	25±3	0.86	26±2	0.96
QX 2	14±1	0.52	15±1	0.58	16±2	0.55	14±1	0.52
QX 3	-	-	-	-	-	-	-	-
QX 4	22±2	0.81	18±3	0.69	22±2	0.76	23±3	0.85
QX 5	20±2	0.74	18±2	0.69	21±2	0.72	22±3	0.81
Ciprofloxacin	27±3	-	26±3	-	29±4	-	27±4	-

All values are Mean±SD; n=3; IZ= Inhibition zone in mm (mean value; include 6 mm diameter of disc), AI= Activity Index (IZ developed by synthesized compound /IZ developed by standard), (-) = No activity

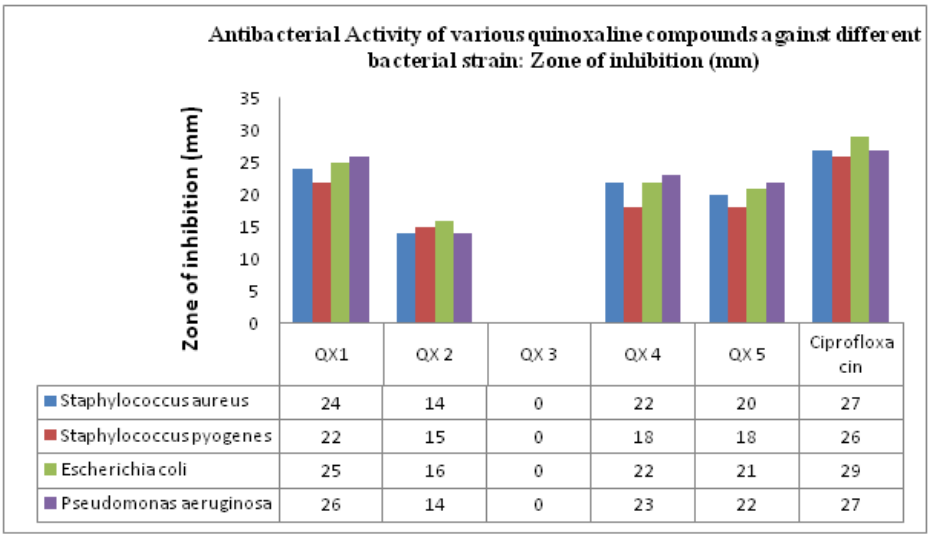


Figure 3: Antibacterial activity of quinoxaline derivatives containing indole ring

Antifungal activity

Table 2: Antifungal activity: Zone of inhibition of the synthesized compounds

Compound / Bacterial strain	Zone of inhibition: Antifungal activity			
	Candida albicans		Aspergilla niger	
Concentration (µg/ml) / IZ and AI	100		100	
	IZ	AI	IZ	AI
QX1	26±4	0.81	14±2	0.88
QX 2	20±2	0.63	12±1	0.75
QX 3	-	-	-	-
QX 4	23±3	0.72	12±1	0.75
QX 5	22±3	0.69	11±1	0.69
Fluconazole	32±4	-	16±2	-

All values are Mean±SD; n=3; IZ= Inhibition zone in mm (mean value; include 6 mm diameter of disc), AI= Activity Index (IZ developed by synthesized compound /IZ developed by standard), (-) = No activity

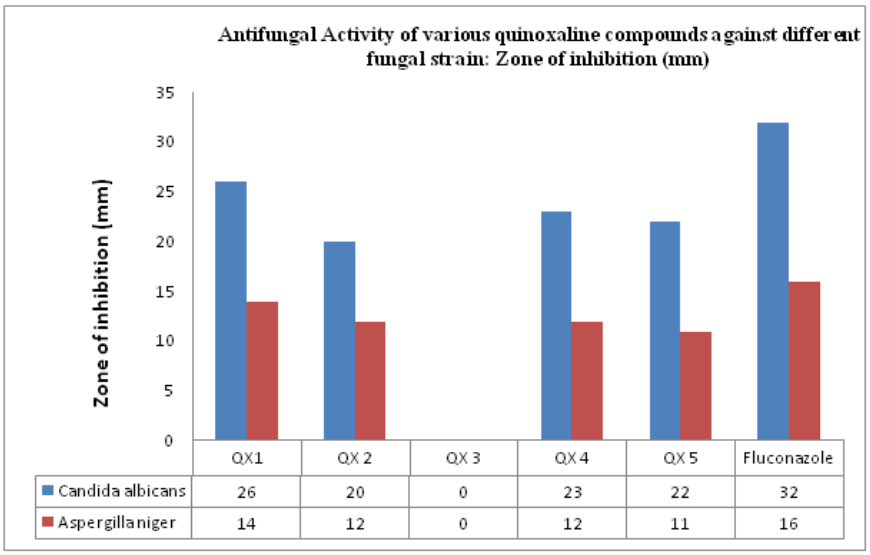
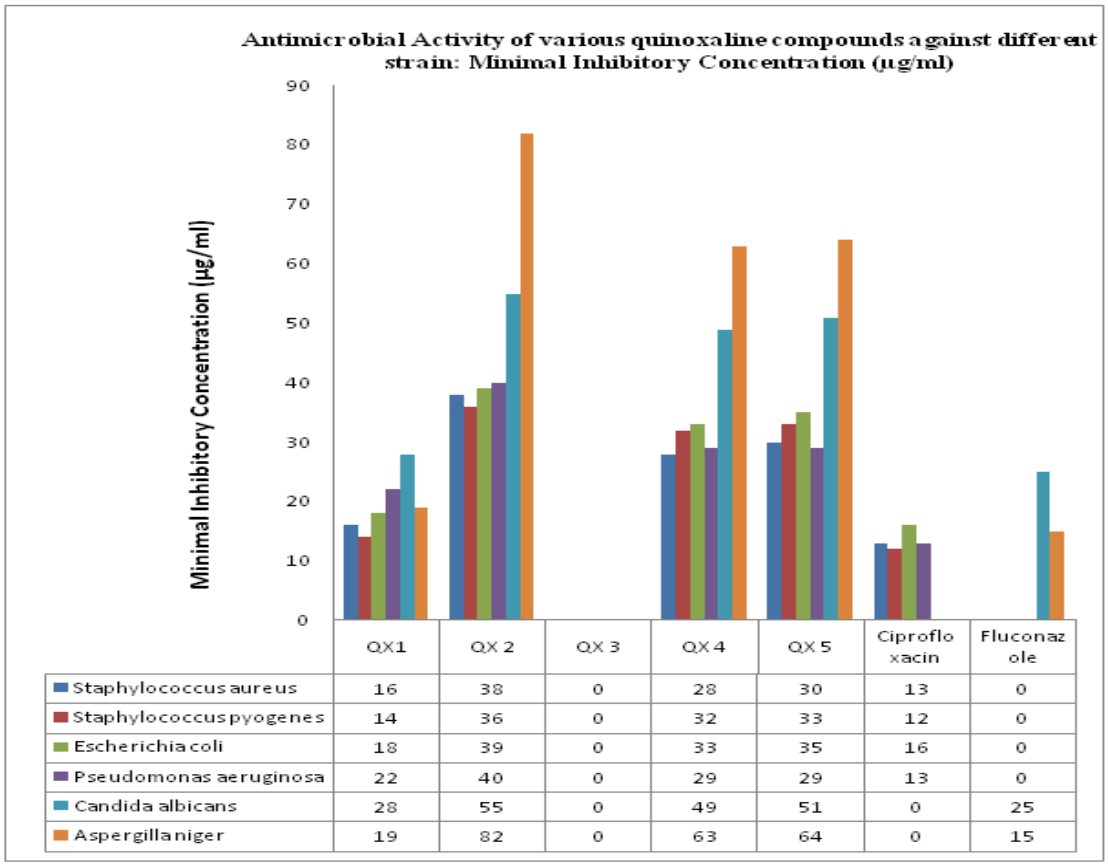


Figure 4: Antifungal activity of quinoxaline derivatives containing indole ring

Determination of MIC:

Table 3: Antimicrobial activity (MIC) of standard & synthesized compounds containing indole ring

Compound	Minimal Inhibitory Concentration (µg/ml)					
	Antibacterial Activity				Antifungal activity	
	Gm +ve		Gm -ve			
Strain	*Staphylococcus aureus*	*Staphylococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillaniger*
QX1	16	14	18	22	28	19
QX 2	38	36	39	40	55	82
QX 3	-	-	-	-	-	-
QX 4	28	32	33	29	49	63
QX 5	30	33	35	29	51	64
Ciprofloxacin	13	12	16	13	NT	NT
Fluconazole	NT	NT	NT	NT	25	15



	QX1	QX 2	QX 3	QX 4	QX 5	Ciproflo xacin	Fluconaz ole
Staphylococcus aureus	16	38	0	28	30	13	0
Staphylococcus pyogenes	14	36	0	32	33	12	0
Escherichia coli	18	39	0	33	35	16	0
Pseudomonas aeruginosa	22	40	0	29	29	13	0
Candida albicans	28	55	0	49	51	0	25
Aspergillaniger	19	82	0	63	64	0	15

Figure 5: Antimicrobial activity of quinoxaline derivatives containing indole ring

Zone of inhibition of the synthesized compounds against various bacterial species are tabulated in table 1 & figure 3. Zone of inhibition of the synthesized of quinoxaline derivatives containing indole ring compounds against two different fungal strain are shown in table 2 and figure 4. Antimicrobial activity (MIC) of standard & synthesized compounds containing indole ring are shown in table 3 and figure 5.

QX1 was found to exhibits the most potent in-vitro antimicrobial activity with the zone Inhibition in mm 24±2 and activity Index 0.89, against *Staphylococcus aureus*, 22±2 and 0.85 against *Staphylococcus pyogenes*, 26±2 and 0.96 against *Pseudomonas aeruginos*, 25±3 and 0.86 against *Escherichia coli* and two fungal strains shown 26±4 and 0.81 against *Candida albicans* and 14±2 & 0.88 against *Aspergilla niger* respectively.

These compounds were screened for their antibacterial activity. The minimum inhibitory concentrations (MICs) of the compounds were determined by agar streak or adopting serial dilution method. Among the synthesized compounds; QX1 was found to exhibits the most potent in-vitro antimicrobial activity with the MICs of 16, 14, 22 and 18 µg/ml against *Staphylococcus aureus, Staphylococcus pyogenes, Pseudomonas aeruginosa, and* Escherichia *coli* respectively. Compound Bromo-1-(4-fluro methyl benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione (QX1) was found to exhibit the most potent in-vitro anti-fungal activity with MICs value of 28 and 19 µg/ml against *Candida albicans* and *Aspergilla niger*.

All substituted quinoxaline compounds have been screened for their antimicrobial activity. From the screening results it was observed that the presence of electron withdrawing group made the substituted quinoxaline compounds to exhibit moderate to significant antibacterial and antifungal activity in comparison to standard drug ciprofloxacin and fluconazole respectively. Compound QX1 and QX4 exhibited promising antibacterial and antifungal activity. However other two compounds (QX5 & QX2) of the series also exhibited moderate to weak activity against the microorganisms as mentioned in table 3 and figure 5.

Therefore compound QX1-QX5 can be recommended for further studies except QX3. The above results recognized the fact that substituted quinoxaline derivatives containing indole ring compounds can be studied further to discover out newer antimicrobial compounds.

In the present study attempt was made to synthesize the derivatives of substituted-1-(4-substituted benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione from p-substituted aniline. Antibacterial activity of synthesized compounds was tested against both gram positive (*Staphylococcus aureus, Staphylococcus pyogenes*) and gram negative (*Pseudomonas aeruginosa, and* Escherichia *coli*) bacteria and the standard drug used for the study was ciprofloxacin. The compounds with two halogen substitution showed better activity.

Some earlier reported researchers also claimed the same as we found and cited above results. Prevalence of fungal diseases has increased significantly in the past 50 years. Fungal diseases manifest themselves differently, including mycoses in the skin, hair, nails, but also as systemic mycoses, being the last one an issue of great medical concern due to the increase in the immunocom promised patient population [21]. One of the most common fungal infections is candidiasis, caused by Candida albicans, a diploid fungus that grows both as yeast and filamentous cells [22]. This fungus can also develop resistance to antimycotic drugs that already exist in the market [23], being important a constant search for new drugs and treatments. Thieno[2,3-d]pyrimidines and pyrrolo[3,4-b]quinoxalines were synthesized and tested against C. albicans, and presented antifungal activity [24, 25]. Researchers also reported some 2-sulphonylquinoxalines and 3-[(alkylthio)methyl]quinoxaline-1-oxide derivatives as compounds with high antifungal activity [26], and also pyrazoloquinoxalines which were observed to be active against fungal infections [27]. The antibacterial activity was screened against Gram-positive Staphylococcus aureus and Bacillus subtilis, and Gram-negative Proteus vulgaris and Klebsiella pneu-moniae, using chloramphenicol as reference drug [28]. Quinoxaline-1, 4-di-*N*-oxide derivatives, pyrazoloquinoxalines and 2-[4- arylidene hydrazinocarbonyl) aniline]-3-methyl quinoxalines have been identified as antibacterial, Antifungal agents and antimicrobial activity [29-33].

CONCLUSION

Starting materials were identified by physical, chromatographic and spectral analysis. The chemical structures of the synthesized compounds were established on the basis of physical, chemical, analytical data. The purification of the compounds was carried by purification methods like recrystallization. Physical constant like melting point, boiling point etc, of the new compounds were determined. The purity and progress of the reactions were monitored by TLC, and column chromatography (if needed) by using suitable solvents and UV, FTIR, NMR, CHN analysis and MASS spectral data were used for the characterization of the synthesized compounds by sending the sample to various advanced research laboratory.

The main objective of the present investigation was to discover newer molecules with potent biological and pharmacological activity such as anti microbial activity on different microbial strain, Analgesic & Anti inflammatory activity on animal model and anthelmentic activity on Pheritma phosthuma and results of all this activities were not included in this article except anti microbial activity. In conclusion, a series of new Substituted-1-(4-substituted benzyl)-1H-indolo (2,3-b) quinoxaline N-benzyl indole-2,3-dione were synthesized successfully with good yield, and were characterized by different spectral studies and their microbiological activity. It was found that moieties have shown potent antimicrobial activity of some compounds containing electron withdrawing substitution like halogen group.

Further combinatorial libraries of these compounds can be produced which can be screened for optimal microbiological activities by optimization techniques using 2D and 3D QSAR investigation.

REFERENCES

1. Bulmer, G. S., "Introduction to medical mycology", 2nd Ed., Benjamin cumiimmgs publishing, London, 2002, pp. 80 – 100.

2. Cruick Shank .R, Duguid. J.P, Marmion.B.P, and swain R.T, "Medicinal Microbiology", 12th edition, churchil Livingstone, London, 1975, pp.196.
3. Iro A., Athina , G., Paola,V., and Franca, Z. "Synthesis and biological evalution of sulfonamide thiazole derivatives as antimicrobial agents", J. chem., Vol.3. No.13, pp.267-273.
4. S. Muralikrishna, P. Raveendra Reddy, L. K. Ravindranath, S. Harikrishna, P. Jagadeeswara Rao, Synthesis Characterization and Biological Evaluation of Novel Thiazole Derivatives Containing Indole Moiety Bearing-Tetrazole, International Journal of Pharma Research & Review, Feb 2014; 3(2):58-64.
5. R.S. Gani, S.R. Pujar and G.S. Gadaginamath; Chemoselective reaction of indole dicarboxylate towards hydrazine hydrate: Synthesis and antimicrobial activity of some new oxadiazolyl methoxyindole derivatives Indian J. Heterocl Chem 2002 12: 25-28.
6. S. Dundappa Danawade, A V Raghu and S .Guru Gadaginamath; Synthesis and antimicrobial activity of novel linearly fused 5-substituted- 7-acetyl-2,6-dimethyloxazolo[4,5-f]indoles. Indian J. Heterocl Chem 2007 46B: 690-693.
7. G. Manjunath Bhovi and S. Guru Gadaginamath; Synthesis and antimicrobial activity of some new 1-[2-phenylthioethyl] 1-[2-phenoxyethyl]-3-ethoxycarbonyl-5-methoxy-2-methylindoles/benz [g] indoles. Indian J. Heterocl Chem 2004 13: 293-296.
8. G.S. Gadaginamath, S.R. Pujar, M.G. Bhovi and C.M. Kamanavalli ; Synthesis and antimicrobial activity of some new 3-acetyl-5-(3-methyl-5-hydroxypyrazol-4-yl) oxy-2-methylindoles. Indian J. Heterocl Chem 2004 13: 209-212.
9. Pandeya SN, Smitha S, Stables JP. Anticonvulsant and sedative–hypnotic activities of N-substituted isatin semicarbazones. Arch Pharm Med Chem 2002; 4: 129-34.
10. Patel A, Bari S, Talele G, Patel J and Sarangapani M. Synthesis and antimicrobial activity of some new isatin derivatives. Iranian J Pharm Research 2006; 4: 249-54.
11. Dorneanu M, Stefanescu E, Paduraru O, Pavelescu M, Hriscu A. The reaction of 3-(R-Phenyl) -6-hydrazine pyridazine with 5-methylisatin and 1-morpholinomethyl-5-methylisatin. Rev Med Chir Soc Med Nat Iasi. 1996; 100 (1-2): 167-71.
12. Bhanupriya Brighu, Devender Pathak, Nadeem Siddiqui, M.Shamsher Alam, Wakaur Ashan. Search for biological activities of Isatin: A short review. 2008; 7(2): 122-25.
13. Bhavesh R Nathani, Kishor S Pandya, Manish M Jeni, Dhimant J Patel and Mayur R Patel, Synthesis and Antimicrobial Activity of Some New Isatins Derivatives, Pelagia Research Library, Der Chemica Sinica, 2011, 2(6):97-103.
14. Anusha Ramgalla, Sharath Chandra R.A.S, Harish Kumar P, Sandeep Ankam, Synthesis and Biological Evaluation of Isatin Derivatives, IJIPSR, 3 (9), 2015, 1305-1318.
15. Chaluvaraju KC, Zaranappa, Synthesis and Biological Evaluation of some Isatin derivatives for Antimicrobial Properties, Research Journal of Pharmaceutical, Biological and Chemical Sciences, January – March 2011, Volume 2, Issue 1,541-546.
16. Rajasree G Pai, Surya M, Muhammed Javahar P B, Subin Mary Zachariah, and Namy George, An Endogenous Heterocyclic Compound Isatin, November – December 2016, RJPBCS, 7(6), 107-120.
17. Hong Min MA, Zhan Zhu LIU, Shi Z, New Apporoach to Synthesis of 6,7 Dimethoxyisatin, 2003; 14: 468-470.
18. Ratnamala P. Sonawane, Rahul R. Tripathi, The chemistry and synthesis of1H-indole-2, 3-dione (Isatin) and its derivatives, Sept 2013, Sci Press Ltd. ILCPA, 7 (1), 30-36.
19. Bhavesh R, Kishor S, Manish M, Mayur R. “Synthesis and antimicrobial activity of some new isatins derivatives”. Scholars Research Library, Der Pharma Chemica, 2011; 3(4): 367-72.
20. Joaquim F. M. da Silva, Simon J. Garden and Angelo C. Pinto. The Chemistry of Isatins: a Review. J. Braz. Chem. Soc., 2001; 12(3): 273-324.
21. S.N.Pandeyaa, D.Srirama, G.Nathb, E De clercqc. Biological activities of isatin and its derivatives. Acta Pharm, 2005; 55 27-46.

22. Tandon, V. K.; Yadav, D. B.; Maurya, H. K.; Chaturvedi, A. K.; Shukla, P. K. Med. Chem. 2006, 14, 6120-6126. DOI: 10.1016/j.bmc.2006.04.029
23. Francis, J.; Landquist, J.K.; levi, A.A.; Silk, J. A. ;Thorpe, J.M. J. Biochem. 1956, 63(3), 455-457.
24. Dirlam, J.P.; Presslitz, J.E.;Williams, B.J. J. Med. Chem. 1983, 26(8),1122-1126.
25. Vicente, E.; Villar, R.; Burguete, A.; et al. Antimicrob. Agents Chemother 2008, 52, 3321-3326.
26. Hanan, R. M.; Ashraf, M. A.; Omneya, K. M. Arch Pharm Res.2004, 11, 1093-1098.
27. Abu-Hashem, A. A.; Gouda, M.A.; Badria, F. A. Eur. J. Med. Chem.2010, 45, 1976-1981. DOI:10.1016/j.ejmech.2010. 01.042
28. Thomas J. Walsh, D.M. D., Spectrum of mycoses, in: S.E. Baron (Ed.), MedicalMicrobiology, fourth ed., 1996.
29. Leah E. Cowen, A. N., Malcolm S. Whiteway, David Y. Thomas, C. Tessier, Linda M. Kohn, James B. Anderson, Proc. Natl. Acad. Sci. U. S. A. 99(14) (2002) 4.
30. Patidar, J. M., A. Mobiya, G. Selvam, Int. J. PharmTech Res. 3 (2011) 386e392.
31. Y.A. Ammar, M.M.F. I., M.S.A. El-Gaby, M.A. Zahran, Indian J. Chem. 41B (2002) 5.
32. Y. Deepika, P. N., K. Sachin, S. Shewta, Int. J. Curr. Pharm. Rev. Res. 1 (3) (2011) 33e46.
33. Ammar et al [24], have synthesized thieno[2,3-d]pyrimidines and pyrrolo[3,4-b]quinoxalines which antibacterial activity was tested against S. aureus and Escherichia coli.