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Editorial Board

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INTERNATIONAL JOURNAL OF PHARMACEUTICAL INNOVATIONS Volume 2, 2014, Issue 5

Invited Article

Part II: Synthesis and Anti-microbial activity of 2-Phenyl-3-substituted- quinazolin-4(3*H*)-ones

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A series of novel 2-phenyl-3-substituted quinazolin-4(3*H*)-one derivative were synthesized by mannich reaction. Newly synthesized compounds were investigated for antimicrobial activity against human pathogenic bacteria and fungi by using cup plate method in nutrient agar medium. The structures of the synthesized compounds were characterized by means of their IR, ^{1}H NMR data. The antimicrobial activity of the new compounds were screened *in vitro* activity against *Streptcoccus aureus* and *Staphylococcus aureus, E.coli* and *Salmonella typhi* and fungus *candida albicans* using amikacin and ketoconazole standard under similar conditions. All the compounds displayed significant anti-bacterial and anti fungal activity. The compound 4-[(4-oxo-2-phenylquinazolin-3(4*H*)-yl-amino]methyl amino benzoic acid (QPAB) was found to be more active derivative in this series. Lead molecule 2-Amino-3-phenylquinazolin-4(3*H*)-one (BN) exhibited antimicrobial activity against Gram (+) and *Candida albicans.* Among these compounds, compounds (QBT, QNF and QMB) exhibited antimicrobial activity against Gram (+) and (–) bacteria and fungus *Candida albicans*.

Keywords: Quinazolin-4(3*H*)-one, Mannich reaction, antibacterial activity, *E coli, S.aureus*

Introduction

Quinazolin-4(3*H*)-one is a novel lead molecule for the design of potential bioactive agents. alagarsamy *et al*., 2000; shah *et al*., 1995; and desai *et al*., 1998 reported anti-HIV activity of 2-phenyl-3-substituted-quinazolin-4-(3*H*)-ones. The literatures also reinforces that the 2-phenyl-3-substituted quinazolin-4(3*H*)-ones also possess to have anti cancer (Girija *et al.,* 2005; Selvam *et al.,* 2006;Manoj *et al.,* 2001; Selvam *et al.,*2004) and marked antiviral (Pandey *et al.,* 1996; Selvam *et al.,*2011) activity against other viruses. A large number of quinazolines have been synthesized and studied for wide range of pharmacological activity but the antimicrobial activities of quinazolines has not been well explored.

Anthranilic acid reacts with benzoyl chloride to form 2-phenyl-1,3-benzoxazin-4-one by *N*-benzoylation followed by dehydrative cyclisation. 2-Phenyl-3-amino quinazolin-4(3*H*)-one derivatives were synthesized by condensation of the compounds containing hydrazine hydrate with 2-Phenyl-1,3-benzoxazine-4-one. A series of

2-Phenyl-3-substituted quinazolin-4(3*H*)-one derivatives were synthesized by condensation of the compounds containing primary aromatic amino group and formaldehyde with 2-Phenyl-3-aminoquinazolin-4(3*H*)-one by mannich reaction (Scheme 1).

Material and Methods

Melting points were determined in open capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded for KBr pellets on a (Shimadzu-8400s) FT-IR spectrophotometer, ^{1}H NMR spectra were determined Bruker AMX 400 MHZ with tetramethyl silane as an internal standard. The sample is dissolved in DMSO-*d*6 and the ^{1}H NMR value is measured in δ ppm.

Synthesis of 2-phenyl-3-substituted quinazolin-4-(3*H*)-one derivatives:

An equimolar (0.01 mol) mixture of quinazoline, different substituents and formaldehyde was refluxed for 6 h with 10 mL of ethanol in acidic condition. The mixture was cooled to room temperature and poured into crushed ice, filter and then washed with water. The solid thus obtained was recrystallised from ethanol. The yield and melting point was predicted in Table-1.

Antibacterial & Antifungal screening

Newly synthesized quinazoiln-4(3*H*)-one derivatives were screened for antibacterial and antifungal activity by cup plate methods using nutrient agar medium (Monica 2010) After sterilization the agar medium was melted, cooled and inoculated with G (+ve) organisms like *Streptcoccus aureus* and *Staphylococcus aureus,* G (-ve) organisms like *E.coli* and *Salmonella typhi* and fungus like *candida albicans* and poured into sterile Petri dish to get a uniform thickness of 5–6 mm. Cups were made out in the other plate using sterile cork borer (6 dm). Then the cups were charged with appropriate concentration of the standard like amikacin (100 µg/ml) and ketoconazole (100 µg/ml). Likewise the cups were also charged with the series of newly synthesized quinazoline derivatives (100 µg/ml) and incubated at 37^{o}C for 24 hours. The diameter of zone of inhibition around the cups were measured and presentated in Table No.1 (Fig 1-3).

Results and discussion

A series of novel 2-Phenyl-3-substituted quinazolin-4(3*H*)-one derivatives were synthesized and investigated for antimicrobial activity by using cup plate method using the nutrient agar medium. The structures of the synthesized compounds were characterized by spectral analysis. The antimicrobial activities of the new compounds were screened against *Streptcoccus aureus* and *Staphylococcus aureus, E.coli* and *Salmonella typhi* and fungus *candida albicans* using amikacin and ketoconazole standard. All the compounds displayed significant anti-bacterial and anti-fungal activity. The compound 4-[(4-oxo-2-phenylquinazolin-3(4*H*)-yl-amino]methyl amino benzoic acid (QPAB) was found to be more active derivative in this series. Among these compounds, compounds (QBT, QNF and QMB) exhibited potent activity against Gram (+) and (–) bacteria and fungus *candida albicans*. Lead molecule 2-Amino-3-phenylquinazolin-4(3*H*)-one (BN) exhibited antimicrobial activity against Gram (+) and *candida albicans* and this lead molecule suitable for further molecular modification.

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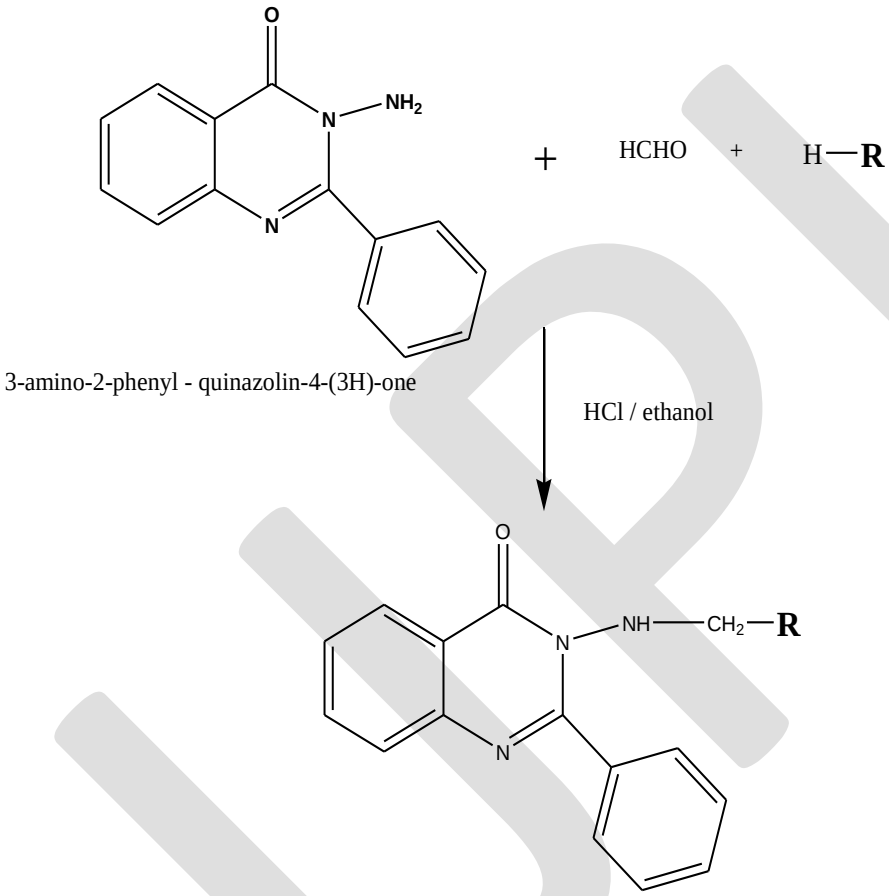
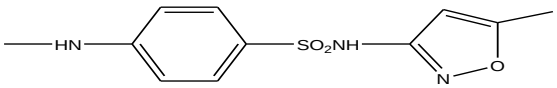
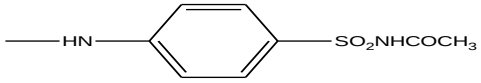
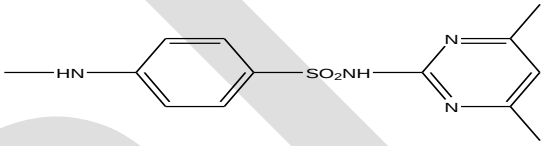
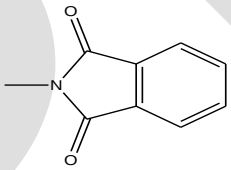
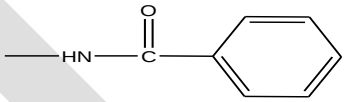
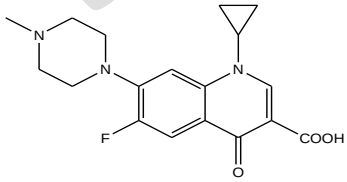
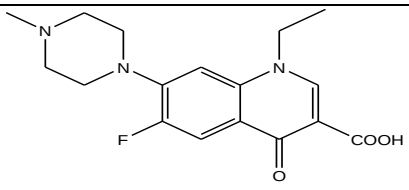
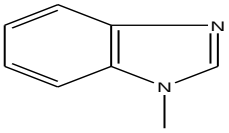
SYNTHESIS OF 2-PHENYL-3-SUBSTITUTED QUINAZOLIN-4(3*H*)-ONES



3-amino-2-phenyl - quinazolin-4-(3H)-one

Compound Code	R
QSM	
QSS	
QSD	
QPH	
QBA	
QCF	
QNF	
QBI	

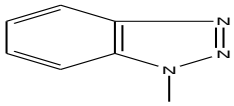
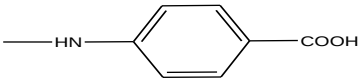
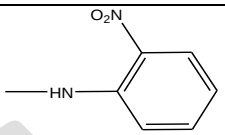
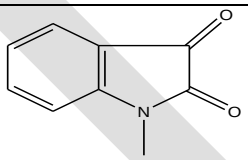
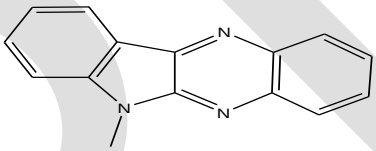
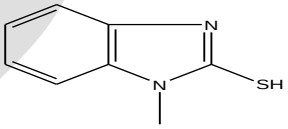
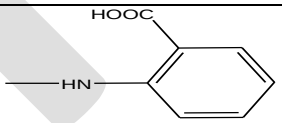
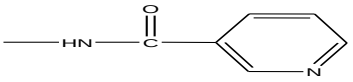
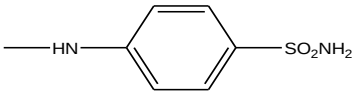
QBT	
QPAB	
QNA	
QIS	
QIP	
QMB	
QAA	
QNI	
QSA	

Fig-1 ANTI-BACTERIAL ACTIVITY OF TITLED COMPOUNDS

AGAINST G (+ ve) ORGANISMS

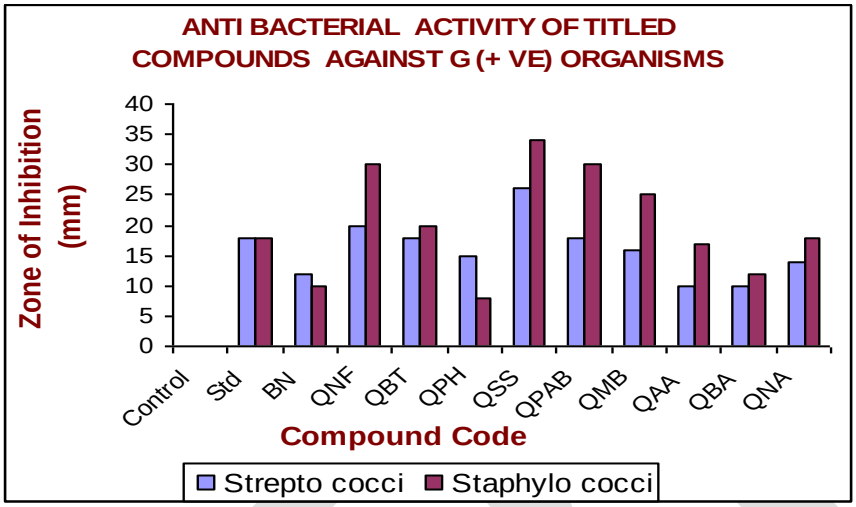


Fig-2 ANTI-BACTERIAL ACTIVITY OF TITLED COMPOUNDS

AGAINST G (- ve) ORGANISMS

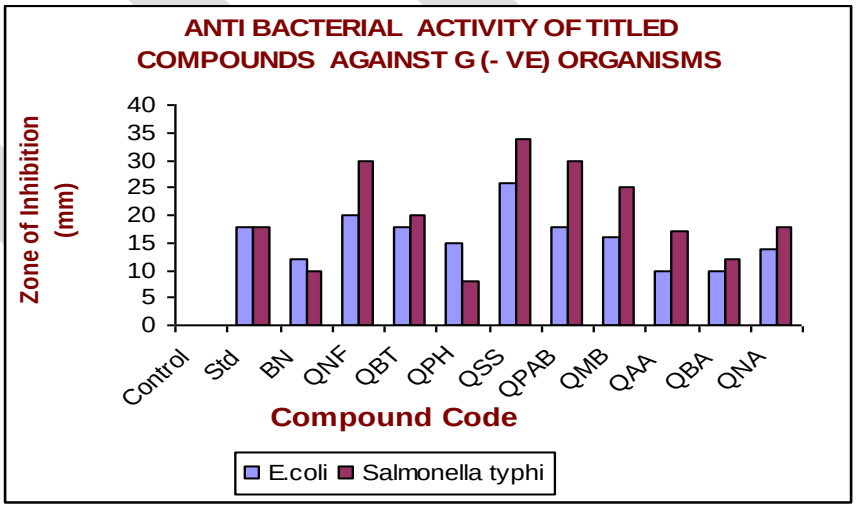


Fig – 3. ANTI-FUNGAL ACTIVITY OF TITLED COMPOUNDS

AGAINST *CANDIDA ALBICANS*

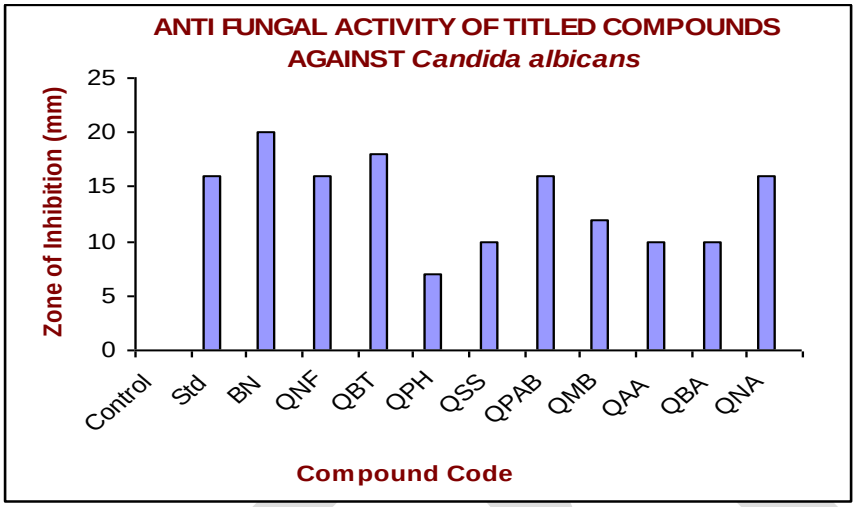


Table 2. Antimicrobial activity of Quinazolin-4(3*H*)-one derivatives

Compounds	Zone of Inhibition (mm)				
	Candida albicans	***Streptococci***	***S. aureus***	***E.coli***	***Salmonella typhi***
BN	20	25	30	12	10
QNF	16	20	30	20	30
QBT	18	30	32	18	20
QPH	07	25	20	15	08
QSS	10	27	30	26	34
QPAB	16	25	26	18	30
QMB	12	20	17	16	25
QAA	10	10	10	10	17
QBA	10	20	17	10	12
QNA	16	21	26	14	18
Control	0	0	0	0	0
Ketoconazole	16	-	-	-	-
Amikacin	-	18	18	18	18

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Research Article

Part II: Synthesis and Anti-HIV activity of Novel Mercaptobenzimidazole Derivatives

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Novel mercaptobenzimidazole derivatives were synthesized by mannich reaction. Mercaptobenzimidazole react with formaldehyde and active hydrogen of sulphonamide (sulphanilamide, sulphacetamide and sulphadimidine) to form *N*-suphanamidomethyl mercaptobenzimidazole derivatives. Structure of synthesized compounds was elucidated by means of spectral (FT-IR, ^{1}H-NMR and MS) analysis. Synthesized compounds were investigated for antiviral activity against HIV-1 and HIV-2 in MT-4 cells and cytotoxicity were also studied in uninfected MT-4 cells by MTT assay. Among the new derivatives evaluated, 4-[(1H-Benzoimidazol-2-ylsulfanylmethyl)-amino]-benzenesulfonamide (MBZ-SN) exhibited cytotoxicity with a CC_{50} of 11.17 µg/mL against mock infected MT-4 cells. They were not active against HIV-1 or -2 for non-cytostatic concentrations.

Keywords: Mercabtobenzimidazole, Sulphanilamide, HIV, MT-4 cells

Introduction

Mercaptobenzimidazole, a versatile lead molecule for potential bioactive agents and its derivatives were reported to possess wide spectrum of activity. Mercaptobenzimidazole derivatives were reported to broad spectrum pharmacological activities (Mohammed *et al.,* 2013). In earlier studies, some novel benzimidazole derivatives were synthesized and evaluated for antiviral and cytotoxicity (Selvam *et al.,* 2010; 2014). In this study we describe the HIV inhibitory activity of some novel of Mercaptobenzimidazole Derivatives (Scheme 1). Synthesized compounds were screened for antiviral activity against HIV 1 and 2 in MT-4 cells and Cytotoxicity were also studied in uninfected MT-4 cells by MTT assay.

Experimental

Melting points were determined using Thomas melting point apparatus and are uncorrected. The purity was checked by TLC using silica gel G as stationary phase. The structure of the synthesized compounds was elucidated using a Perkin Elmer FT-IR in KBr disc and PMR was taken on a Bruker AMX-(400 MHz) FT-NMR. Mass spectra were obtained on a Varian Atlas CH-7 Mass spectrometer at 70 eV.

Synthesis of N-Sulphanoamidomethyl-Mercaptobenzimidazole

Equimolar quantities (0.01 mol) of mercaptobenzimidazole, formaldehyde (37%) and sulphonamide (sulphanilamide, sulphacetamide and sulphadimidine) were dissolved in warm ethanol. The reaction mixture was stirred in magnetic stir at room temperature for 3 hours and kept in refrigerator overnight. The resultant solid was washed with dilute ethanol and recrystallized from ethanol-chloroform mixture (Scheme 1). The structure of synthesized compounds were elucidated by spectral analysis

4-[(1H-Benzoimidazol-2-ylsulfanylmethyl)-amino]-benzenesulfonamide(MBZ-SN): yield: 62 %, mp: 276^0, IR (KBr) in Cm^{-1} 3350 (NH), 1620 (C=C), 1580 (C=N), 1160 (SO_2); ^1H NMR (DMSO-d_6) in δ ppm 2.5 (s, 1H, NH-Benzimidazole) 4.6 (s,2H,-NCH_2 N-) 6.7-7.8 (m, 9H, Ar-H), 10 (b, 1H, - SO_2 NH-) EIMS (M/z) 334.

***N*-Acetyl-4-[(1H-benzoimidazol-2-ylsulfanylmethyl)-amino]-benzenesulfonamide(MBZ-SAC):** yield: 68 %, mp: 210^0, IR (KBr) cm^{-1}: 3320 (NH), 1720 (C=0), 1695 (C=N), 1540 (C=C), PMR (DMSO-d_6) δ ppm: 2.02 (s, 3H, -CH_3), 2.5 (s, 1H, NH-Benzimidazole) 4.6 (s,2H,-NCH_2 N-) 6.7-7.9 (m, 9H, Ar-H) 10 (b, 1H, - SO_2 NH-); EI-MS (m/e):376

4-[(1H-Benzoimidazol-2-ylsulfanylmethyl)-amino]-*N*-(4,6-dimethyl-pyrimidin-2-yl)benzenesulfonamide (MBZ-SDM): yield: 60 %, mp: 242^0, IR (KBr) cm^{-1}: 3320 (NH), 1690 (C=N), 1587 (C=C), PMR (DMSO-d_6) δ ppm: 2.04 (s, 6H, -2XCH_3), 2.5 (s, 1H, NH-Benzimidazole) 4.6 (s,2H,-NCH_2 N-) 6.7-7.9 (m, 9H, Ar-H), 8.2 (d, 1H, pyrimidinyl-H) 10 (b, 1H, - SO_2 NH-); EI-MS (m/e): 440

Anti-HIV assay

The compounds were tested for anti-HIV activity against the replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells (Selvam *et al.,* 2003). The cells were grown and maintained in RPMI 1640 Medium supplemented with 10% heat–inactivated Fetal Calf Serum (FCS), 2mm- glutamine, 0.1% Sodium bicarbonate and 20 μgm/ml gentamicin (culture medium). HIV-1 (HTLV-IIIB/LAI) strain and HIV-2 ($LAV\text{-}2_{ROD}$) strain were used in the experiment. The virus strains were propagated in MT-4 cells. Titer of virus stock was determined in MT-4 cells and the virus stock was stored at -70°C until used.

Inhibitory effects of the compounds on HIV-1 and HIV-2 replications were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by MTT assay. Briefly, 50 µl of HIV-1 or HIV-2 (100-300 $CCID_{50}$) and MT-4 cells were added at a final concentration of 6x105 cells/ml were added to flat-bottomed MT–4 wells. After 5^{th} day of incubation at 37°C the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. Cytostatic activity of the extracts for mock- infected MT-4 cells was also assessed by the MTT method. Anti-HIV activity and cytotoxicity of standard AZT were also performed by a similar method in MT-4 cells. The anti-HIV data are presented in Table 1.

RESULTS AND DISCUSSION

Novel mercaptobenzimidazole derivatives were synthesized by mannich reaction. Mercaptobenzimidazole react with formaldehyde and active hydrogen of sulphonamide (sulphanilamide, sulphacetamide and sulphadimidine) to form *N*-suphanamidomethyl mercaptobenzimidazole derivatives. Their chemical structure was elucidated by means of spectral (FT-IR, ^{1}H-NMR, MS) analysis. Synthesized compounds were investigated for antiviral activity against HIV-1 and HIV -2 in MT-4 cells and Cytotoxicity was also studied in uninfected MT-4 cells by MTT assay. All the compounds exhibits cytotoxicity against MT-4 cells (C-type Adult T Leukemia Cells) with CC_{50} value of 11-56 µg/mL Among the new derivatives evaluated, 4-[(1H-Benzoimidazol-2-ylsulfanylmethyl)-amino]-benzenesulfonamide(MBZ-SN) exhibited significant cytotoxicity with an CC_{50} of 11.17 µg/mL against mock infected MT-4 cells. They were not active against HIV-1 or HIV -2 for non-cytostatic concentrations. This class of compounds suitable for designing of potential inhibitors of WNV NS3 Protease (Selvam *et al.*, 2014).

Acknowledgement:

Authors expressed wholehearted thanks to Rega Institute for Medical Research, Katholike University, Leuven, Belgium for anti-HIV activity

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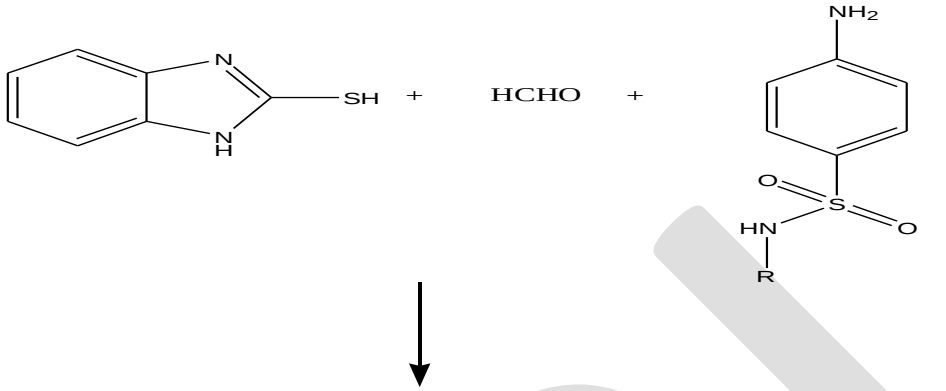
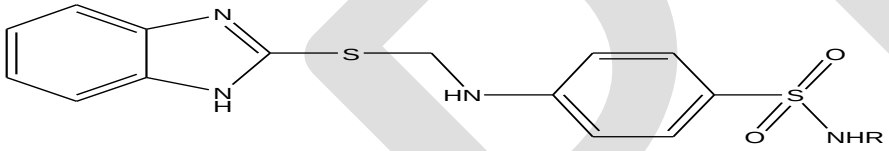
Table 1. Anti-HIV activity and Cytotoxicity of Inhibitory Mercaptobenzimidazole

Compounds	Strain	IC_{50} [a], (μg/ml)	CC_{50} [b](μg/ml)	Maximum Protection
MBZ-SN	IIIB	>11.17	11.17±0.64	2
	ROD	>11.17	11.17±0.64	4
MBZ-SAC	IIIB	>56.70	56.70±4.86	2
	ROD	>56.70	56.70±4.86	3
MBZ-SDM	IIIB	>12.30	12.30±0.26	2
	ROD	>12.30	12.30 ±0.26	3
AZT	IIIB	0.0015 ±0.0002	>25	95
	ROD	0.0016±0.0003	>25	75

[a]Effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV. [b]50% Cytotoxic concentration of compound, required to reduce the viability of mock infected MT-4 cells by 50%.

Scheme 1. Synthesis of N-Sulphanoamidomethyl-Mercaptobenzimidazole





Where	R
MBZ-SN	H
MBZ-SAC	$-COCH_3$
MBZ-SDM	-2,6-Dimethylpyrimidine

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Short communications

Studies on cytotoxicity of *Wrightia tinctoria Leaf extracts against Liver cancer cells*

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Wrightia tinctoria **(WT) leaf extracts were investigated for cytotoxic effect on HepG2 (Human Liver Cancer) cell culture by MTT assay. Chloroform and n-butanol extracts (CWT and BUWT) showed cytotoxicity against HepG2 cells with CTC_{50} (cytotoxicity 50%) values of 125 and 90 µg/ml, where as standard Cis platin was found to be 11.09 µg/ml. chloroform extract (CWT) showed significant cytotoxicity against HepG2 cells and CWT merits for further investigation to screen its anti-cancer activity using *in vivo* models**.

Keywords: *Wrightia tinctoria,* HIV Integrase activity, Aqueous extract

INTRODUCTION

Wrightia tinctoria is an important medicinal plant used in the Indian system of medicine for the treatment of variety of diseases[8] (Bigoniya *et al.*, 2008) and it reported to possess analgesic (Reddy et al., 2002) antifertility (Keshri *et al.,* 2008), cytotoxic (Kawamoto et al., 2003) hemostasis (Rajesh *et al.,* 2003), anti-ulcer activity (Bigoniya *et al.,* 2006) and Hepatoprotective (Selvam et al., 2011). Indole derivatives such as isatin, indirubin, indigotin and tryphanthrin are the principle active constituents of *wrightia tinctoria* (Muruganandhan *et al.,* 2000) which may responsible for board spectrum biological activity. Review of literature revealed that cytotoxicity of *Wrightia tinctoria* against the human liver cancer cells Hep G2 cells is relatively less explored. The present study is designed to find the inhibitory activity of leaf extracts of *Wrightia tinctoria* (WT) against the human liver cancer cells Hep G2 cells.

Extraction: Leaf parts of *Wrightia tinctoria* (Apocynaceae) were collected in and around Tenkasi, Tamilnadu, India and were dried in shade, subjected to hot continuous percolation using chloroform and n-butanol. chloroform (CWT) and n-butanol (BUWT) of *Wrightia tinctoria* were concentrated by distillation and dried under vaccum.

Preparation of suspensions

Methanolic extract and isolated compounds of Noni fruits (*Morinda citrifolia* L) dissolved in DMSO and the volume was made up to 10 ml with DMEM/MEM to obtain a stock solution of 1mg/ml concentration and stored at -20°C prior to use. Further dilutions were made to obtain different concentrations ranging from 1000–62.5 µg/ml with respective media and used for i*n vitro* investigations.

Cell lines and growth media

HepG2 (Human liver cancer) cells were cultured in MEM (Minimum Essential Medium) and DMEM (Dulbecco's Modified Eagles Medium) respectively. The medium also contains 10% fetal calf serum, penicillin (100 IU) and streptomycin (100 µg).

***In vitro* cytotoxicity screening**

The ability of the cells to survive a toxic insult is the basis of most cytotoxicity assays (Philips *et al.,* 1990). The monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtiter plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours partial monolayer was formed, the supernatant liquid was flicked off washed the monolayer once and 100µl of different drug concentrations were added to the cells in microtiter plates. The plates were then incubated at 37^oC for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and MTT assay performed.

RESULTS AND DISCUSSION

The *Wrightia tinctoria* (WT) leaf extracts were investigated cytotoxic effect on HepG2 (Human Liver Cancer) cell culture by MTT assay. Chloroform and n-butanol extracts (CWT and BUWT) showed cytotoxicity against HepG2 cells with CTC_{50} (cytotoxicity 50%) values of 125 and 90 µg/ml, where as standard Cis platin was found to be 11.09 µg/ml. chloroform extract (CWT) showed significant cytotoxicity against HepG2 cells and CWT

merits for further investigation to screen its anti-cancer activity using *in vivo* models. Indirubin-3'-monoxime (Heredia *et al.*,2005) a derivative of a Chinese antileukemia medicine *isatis tinctoria* inhibits HIV-1 replication and another isatin containing medicinal plant *Isatis indigotica* also reported to posses anti HIV activity (Liu *et al.*, 2007). Anti-HIV and Cytotoxicity of *Wrightia tomentosa* also demonstrated by our preliminary studies (Selvam *et al.*, 2012). To our best knowledge, this is the first time to report the cytotoxic effect on HepG2 (Human Liver Cancer of these Indian plants locally known as *wrightia tinctoria*. The isolation of active compounds possessing invitro anti-cancer from *wrightia tinctoria* are now in progress.

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Table 1. Determination of CTC_{50} by using MTT assay in HepG2 cells

Extract	CTC_{50}* value (in µg/ml)
CWT	**125 ± 11.71**
BUWT	**190± 20.23**
Cis Platin (STD)	**11.09 ±0.59**

*Cytotoxic 50% concentration,

*Average of four independent determinations, values is mean ± S.E.M.

Synthesis, Characterization and Antibacterial evaluation of Quinoxaline derivatives

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A series of *N*-(4*H*-imidazo[4,5-b]quinoxalin-2(9*H*)-ylidene)substituted amine **(QX3-QX5)** was designed, synthesized and characterized for evaluation of potential antibacterial activity screened against gram negative viz *Escherichia coli, Klebsella pneumonia* and gram positive bacteria viz *staphylococcus aures* and *Bacillus subtilis*. Ciprofloxacin used as standard drug. Structure activity relationship led to the conclusion, all the compounds showed potent to moderately potent antibacterial activity. Among the compounds **QX-1, QX-2, QX-5** showed potent antibacterial activity and **QX-3** & **QX-4** showed less antibacterial activity. Therefore maximum compounds of this series can serve as a lead molecule for further development as new class of antibacterial agent.

Introduction

Heterocyclic ring system of Quinoxaline and their derivatives aroused great interest for the past and recent years due to wide variety of biological properties such antiviral (Periyasamy Parthiban *et al.,* 2014), anti-inflammatory (Richards GJ *et al.,* 1979), antimicrobial activity (John B et al., 2003) , anticancer and antitubercular (Alagarsamy *et al.,* 2011) and anticonvulsant (Rajashegaran A *et al.,* 2005) activities. Based on literatures review we have planned to an attempt was made to synthesize some new quinoxaline derivatives with a mixture of Oxalic acid, ortho phenylene diamine and glacial acid was heated to reflux. A new series of quinoxaline derivatives QX-3 to QX-5 have been synthesized from the combination of QX-2 and substituted amines which is screened for antibacterial activity. These compounds have been characterized by IR, ^{1}HNMR and Mass spectrum. The title compounds were synthesized by the following synthetic route depicted in **Scheme I**.

EXPERIMENT

Melting points (mp) were determined in open capillary tubes on Thomas Hoover melting point apparatus and are uncorrected. The IR spectra were recorded in film or in potassium bromide disks using a Perkin-Elmer 398 spectrometer. The ^{1}H NMR spectra were recorded on DPX-500 MHz Bruker FT-NMR spectrometer. The chemical shifts were reported in parts per million (δ ppm) relative to TMS as an internal reference. Mass spectra were recorded on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). Elemental analysis (C, H and N) was performed on a Perkin-Elmer 2400 analyzer and values were within the acceptable limits of the calculated values. The progress of all the reactions were monitored by readymade silica gel plates (Merck) and a solvent system of chloroform-methanol (9:1). The spots were developed in iodine chamber.

Spectral data (IR, NMR and Mass) was confirmed the structures of the synthesized compounds and the purity of these compounds were ascertained by microanalysis. Elemental (C, H and N) analysis indicated that the calculated and observed values were within the acceptable limits. All chemicals and reagents were procured from Aldrich (USA), Lancaster (UK) or Spectrochem Pvt.Ltd (India) and were used without further purification.

Step 1: Synthesis of Compound QX-1 (Quinoxaline-2,3(1H,4*H*)-dione): A mixture of equimolar concentrations of O-phenylene diamine **(1),** Oxalic acid **(2)** and 5ml of Glacial acetic acid **(3)** was heated to reflux. Then the solution was poured into ice cold water. The crystalised product is obtained by filteration. **Compound QX 1:** Mol.formulea: $C_8H_6N_2O$; Yield: 92%, mp: 360-362^0 C, IR (KBr) cm^{-1}: 1655 (C=O), 1711 (C=O), 3058 (NH), 3368 (NH), ^{1}NMR ($CDCl_3$) δ ppm: 7.2-7.6 (m, 4H, Ar-H),7.8 (s, 1H, Ar-NH) 8.2 (s,1H, Ar-H) EI-MS (m/e):162.

Step 2: Synthesis of Compound QX-2 (4*H*-imidazo [4,5-b]quinoxalin-2(9*H*)-one): A mixture of QX-1 and urea **(4)** is condensed and was heated on refluxing. The process of the reaction was monitored by TLC and reflux was continued for few hours until it forms clear solution. After cooling to room temperature, the reaction mixture was poured into cold water, extracted with dicholoro methane and was recrystallized with ethanol. **Compound QX-2:** Mol.formulea: $C_9H_6N_4O$; Yield: 92%, mp: 274-276^0 C, IR (KBr) cm^{-1}: 1655 (C=O), 1701 (C=O), 3293 (NH), 3366 (NH), ^{1}NMR ($CDCl_3$) δ ppm: 7.2-7.4 (m, 4H, Ar-H), 8.2 (s, 1H, Ar-NH) 8.4 (s,1H, Ar-NH) EI-MS (m/e):186.

Synthesis of Compound QX3-QX5: Compound QX-3 and substituted anilines **(5)** were taken in equimolar quantities and refluxed with sufficient amount of ethanol in presence of 2-3 drops of glacial acetic acid. The completion of reaction was monitored by TLC. The product obtained was recrystallised with ethanol. Compound QX4 (final compound): Mol.formulea: $C_{15}H_{11}N_5$; Yield: 88%, mp: 208-210^0 C, IR (KBr) cm^{-1}: 3060 (NH), 3342 (NH), 1262 (C=N), 3642 (Ar-OH), ^{1}NMR ($CDCl_3$) δ ppm: 6.8-7.2 (m, 8H, Ar-H), 7.9-8.1 (s, 2H, Ar-NH), 2.62 (s,1H,OH) EI-MS (m/e): 261.

BIOLOGICAL

The novel title compounds were screened for their antibacterial by disc plate method (Periyasamy Parthiban *et al.*,2014, and Periyasamy Selvam *et al.,* 2011). The test organism was subcultured by using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1ºC for 24 hours, they were stored in the refrigerator. The stock solutions were maintained. Bacterial inoculums were prepared by transferring a loopful of stock solution to nutrient broth. The flask was incubated at 37±1ºC for 48 hours before the experimentation. Antibacterial activity screened against gram negative viz *Escherichia coli, Klebsella pneumonia* and two gram positive bacteria i.e *staphylococcus aures* and at *Bacillus subtilis*. The test organism was a two hour culture of *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli* incubated and grown in pepton-water medium (Temp 37**º**C). Dimethyl sulphoxide (DMSO) was used as solvent control which did not show any inhibition. Antibacterial activity was determined by measuring the diameter (mm) of the inhibition zone at minimum level concentration. Ciprofloxacin 50µg/ml was used as standard drug for antibacterial activity. Solutions of the test compounds QX-1 to QX-5 were prepared by dissolving 10mg each in Dimethyl sulphoxide (DMSO, 10ml). A reference standard for gram positive and gram negative bacteria was made by dissolving accurately weighed quantities of drug (30µg/ml). The nutrient agar medium was sterilized by autoclaving at 121ºC (15 lb/sq.inch) for 15 min.

RESULTS AND DISCUSSION

CHEMICAL

Three quinoxaline derivatives were synthesized by simple reaction. In the first step, a mixture of equimolar concentrations of O-phenelene diamine and Oxalic acid and Glacial acetic acid was heated to reflux. **(3)**. Compounds QX3-QX5 was prepared by reflux method. The title compounds of IR spectrum were indicating that disappearance of C=O peak of starting material and appearance of C=N peak due to substituted amines at 1262 cm^{-1} of *N*-(4*H*-imidazo[4,5,-b]quinoxaline-2-(9*H*)-ylidene)benzenamine **(QX-4)**. The ^{1}HNMR spectrum showed a a singlet for (OH) around at δ 2.62 and multiplet for aromatic protons (8H) in the range between δ 7.9-8.1 ppm.

BIOLOGICAL

The novel title compounds **(QX-3-QX-5)** were screened for their antibacterial by measuring the MIC & Zone of inhibition. The zone of inhibition of various concentrations of synthesized compounds showed better activity against *Bacillus subtilis*. All the compounds showed potent to moderately potent antibacterial activity against other bacteria's by zone of inhibition method. Among the compounds QX-1, QX-2 & QX-5 compounds showed antibacterial activity. QX-3 and 4 showed less antibacterial activity. The results are given in **Table 01**. The MIC was measured for all test compounds; the test compounds showed better activity against *Klebsiella pneumonia* and *Staphylococcus aures.* The compounds are not inhibited against *Escherichia coli*. It indicates that less activity. The antibacterial activity of test compounds showed moderate activity against *Bacillus subtilis.* The results are shown in **Table 02**.

Conclusion

In the present study certain quinoxaline derivatives were synthesized based on literature some of the synthesized compounds showed characteristic peaks in IR spectra. Antibacterial studies were performed for all the synthesized compounds (30µg/ ml) and compared to standard ciprofloxacin (50µg/ ml). The zone of inhibition & MIC of various concentrations of synthesized compounds against gram positive and gram negative bacteria was measured. All the compounds showed potent to moderately potent antibacterial activity. Among the compounds QX-1, QX-2, QX-5 compounds showed antibacterial activity. QX-3 and QX-4 showed less antibacterial activity.

ACKNOWLEDGEMENT

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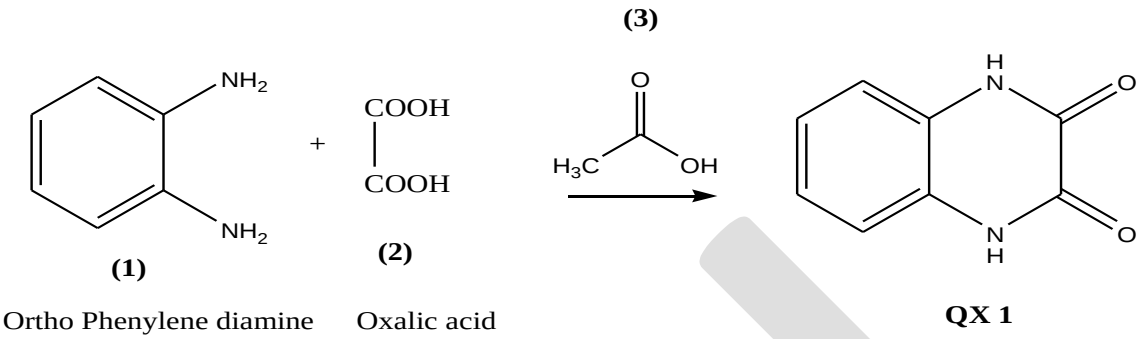
Scheme I: The title compounds were synthesized by the following synthetic route depicted as follows,

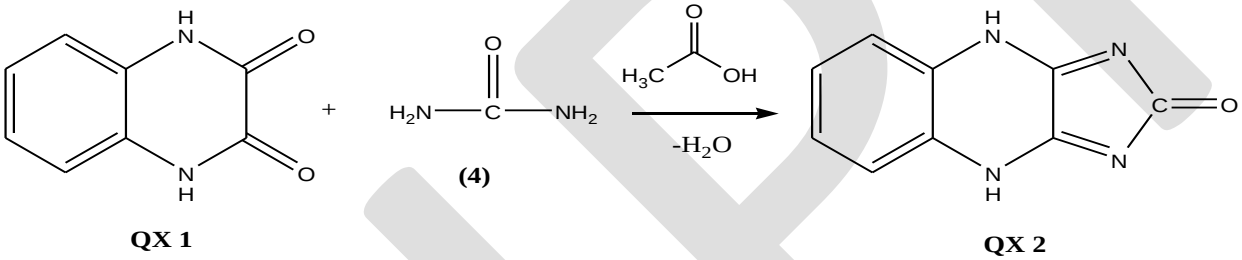
Step-I



(1) **(2)** **QX 1**

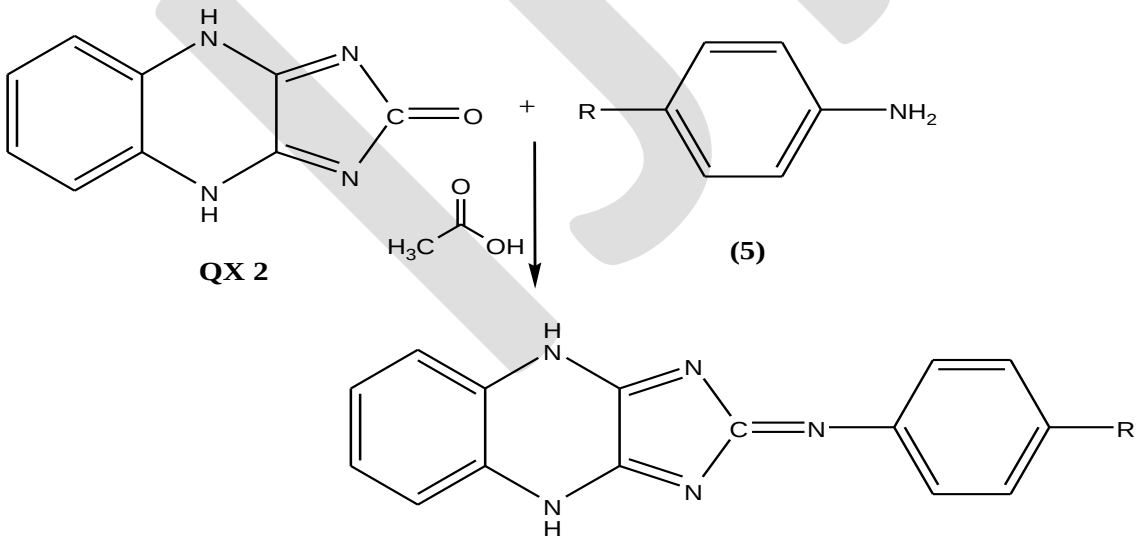
Ortho Phenylene diamine Oxalic acid

Step-II



QX 1 **(4)** **QX 2**

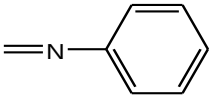
Step-III

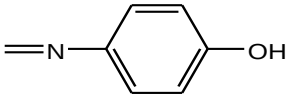


QX 2 **(5)**

QX 3-QX 5

R,

QX 3; 

QX 4; 

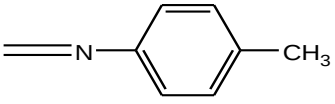
QX 5; 

Table 01; Zone of inhibition of synthesized compounds against all bacteria

Test Compounds	Concentrations (µg/ ml)	Zone of inhibition (mm)											
		B.subtilis			*S.aureus*			*K.pneumonia*			*E.coli*		
		25	50	100	25	50	100	25	50	100	25	50	100
QX-1	Concentration (30µg/ ml)	7	13	23	8	12	21	9	11	2	6	12	15
QX-2		6	13	23	8	12	21	9	11	20	6	12	15
QX-3		12	23	25	15	18	26	12	19	22	10	11	22
QX-4		9	18	22	12	18	20	8	11	17	6	17	23
QX-5		12	16	22	6	11	18	6	16	18	6	11	15
Standard	Ciprofloxacin (50µg/ ml)	20			21			23			22		

Table 02; MIC of synthesized compounds against all bacteria

S.No	Test Compounds	Minimum inhibitory concentration (MIC) (µg/ml)			
		B.subtilis	*S.aureus*	*K.pneumonia*	*E.coli*
1	QX-1	23	24	23	22
2	QX-2	21	25	23	21
3	QX-3	22	23	23	24
4	QX-4	23	22	24	22
5	QX-5	24	21	22	23

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A short introduction of the research problem followed by a brief review of literature and objective of the research.

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