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ANTIMICROBIAL AND ANTI-INFLAMMATORY ACTIVITY OF SCHIFF-BASE DERIVATIVES CONTAINING BENZIMIDAZOLE MOIETY

Irfan N. Shaikh[a,*], Umar Farooq Bagwan[a], Mahantesh M. Kurjogi[b], Kallappa M. Hosamani[c],

[a]Department of Chemistry, SECAB Institute of Engineering & Technology, Vijayapur–586101, India.
[b]Department of Biotechnology and Microbiology, Karnatak University Dharwad-580 003, India
[c]P.G. Department of Studies in Chemistry, Karnatak University Dharwad-580 003, India.

***Corresponding Author: Irfan N. Shaikh**

Department of Chemistry, SECAB Institute of Engineering & Technology, Vijayapur–586101, India.

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ABSTRACT

A new class of Schiff base derivatives containing benzimidazole moiety were screened for their potential antimicrobial and anti-inflammatory properties. Some of these derivatives showed moderate to potent *in-vitro* antimicrobial and *in-vivo* anti-inflammatory activities. The results revealed that, Compound **a12** and **a20** exhibited the most potent antibacterial activity with lowest MIC value against *S. aureus* and *E. coli*. While compounds **a12**, **a14**, **a17**, **and a18** showed excellent antifungal activity when compared with standard drugs. Compounds **a12**, **a14**, **a15, a17, a18 and a20** were found to be the main structural requirement for maintaining anti-inflammatory activity.

KEYWORDS: Schiff's Base, Benzimidazole derivatives, Antimicrobial, Anti-inflammatory.

INTRODUCTION

The treatment of infectious diseases still remains an important and challenging problem caused by a combination of factors; among them emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. There is a real perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms, which are distinct from those of well-known classes of antibacterial agents, to which many clinically relevant pathogens are now resistant. Through various molecules designed and synthesized for this aim, it was demonstrated that benzimidazole and its derivatives could be considered as possible antimicrobial agents (Zhang et al, 2009; Goker et al, 2002).

Inflammation is a normal response to any noxious stimulus that threatens the host and may vary from a localized response to a generalized one (Williams and Lemke, 2002). Non-steroidal anti-inflammatory drugs (NSAID) are one of the most widely used drug category against inflammation, mild to moderate pain, and fever. Specific uses also include the treatment of headaches, arthritis, sports injuries, and menstrual cramps. Their use is mainly restricted by their well known and serious adverse gastrointestinal side effects such as gastroduodenal erosions and ulcerations (Chan, 2006; Whittle, 2005; Cryer, 2003; Bjorkman, 1996; Kimmey, 1992). NSAID-induced gastropathy are estimated to affect up to half of chronic NSAID users, with major world health implications (Richy et al., 2004). Therefore, search for better and safer anti-inflammatory agents is always going on at a rapid pace.

Benzimidazole is one of the most promising heteroaryl moieties that yielded many successful drugs (Preston, 1980). Derivatives of these compounds are known for their antibacterial (Sharma et al., 2006), antifungal (Kus and Altanlar, 2003) activities. The success with these compounds stimulated the search for new biologically active derivatives. Some of these compounds exhibited anticancer (Kruse et al., 1989), antiviral (Jun et al., 2005), antihelmentic (Anelia et al., 2006), anti-inflammatory (Labanauskas et al., 2000), antihypertensive (Kubo et al., 1993) and anticoagulant agents (Mederski et al., 2004). These derivatives also exhibit significant activity against several viruses such as HIV (Porcari et al., 1998; Roth et al., 1997).

On the other hand, Schiff bases derived from aromatic amines and aromatic aldehydes have a wide variety of applications in many fields, *e.g.*, biological, pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers (Cimerman et al., 2000; Dhar and Taploo, 1982). They are reported to show characteristic biological activities including antibacterial, antifungal, antitumor, anticancer, anti-convulsant, anti-

inflammatory and analgesic activities (Deepa et al., 2008; Mohsen et al., 2010; Rajyalakshmi et al., 2011; Chinnasamy et al., 2010; Sham et al., 2006). Other application of Schiff's bases includes industrial synthesis of high value life saving beta lactam (Taggi et al., 2002) antibiotics from class of penicillins and cephalosporins. Considering extensive applications of benzimidazole moiety in medicinal chemistry and in continuation of our ongoing project on biologically active heterocycles (Shingalapur et al., 2009; Hosamani et al., 2009; Hugar et al., 2010), an attempt has been made to evaluate *in-vitro* antimicrobial and *in-vivo* anti-inflammatory activities.

RESULTS AND DISCUSSION

2.1. Chemistry

The synthesis of Schiff's base from substituted 3-(1H-Benzimidazol-2-yl) naphthalene-2-amine and various aldehydes is reported in the literature (Varsha et al., 2011).

1.2. Pharmacology

All the compounds prepared herein were screened for their potential *in-vitro* and *in-vivo* biological activities such as antimicrobial, anti-inflammatory and anticonvulsant activities. Minimum inhibitory concentration (MIC) of newly synthesized bis-benzimidazole derivatives were evaluated against three human bacterial pathogens viz. *S.aureus*, *E.coli*, *Salmonella typhi* and fungal pathogen viz. *Candida albicans*, by disc diffusion method (Bauer et al., 1966). The compound at the concentration range (10, 15, 20, 25 and 30 µg/mL) in DMSO was used in this study with Chloramphenicol and Penicillin respectively, for bacteria and Ampoterecin B and Fluconazole for fungi being used as control. Anti-inflammatory activity was evaluated using carrageenan induced hind paw oedema method (Winter et al., 1962).

1.2.1. Antimicrobial activity

The investigation of antibacterial screening of all the tested compounds **(a11–a20)** against both gram positive and gram negative human pathogenic bacterial strains showed moderate to excellent activity and was compared with the standard antibiotics like Chloramphenicol and Penicillin. Screening results are summarized in **Table 1.** The best antibacterial effect has compounds **a12** and **a20** with MIC –10µg/mL against *S.aureus*, *E.coli* and 15µg/mL against *Salmonella typhi*. According to the Structure–activity relationship of the antibacterial activity, compounds **a12** and **a20** is greatly influenced on introduction of the electron–withdrawing effect of chlorine atoms, which are directly attached to the phenyl ring. In contrast with standards, attachment of a chlorine atom at the *ortho* and *meta* position of the phenyl ring, exemplified by compounds **a12**, **a14**, **a18** and **a20** are more effective than the tested antibiotics viz., Chloramphenicol and Pencillin with MIC – 15 and 25µg/mL against *S.aureus* (**Table 1**). Unfortunately, **a11**, **a15**, **a16** leads to weak antibacterial activity against Gram negative bacteria *S.aureus*. Similarly **a13**, **a14**, **a16, a17** and **a19** showed better activity against *E. coli*. Whereas, except **a11**, **a15**, **a16** and **a19** all the compounds were active against *Salmonella typhi*. We can also notice that the exerted action on *S.aureus*, *E.coli* is better than *Salmonella typhi*.

The antifungal activity was evaluated against fungal strain such as *Candida a*. Ampoterecin B and Fluconazole were used as a standard for the comparison of antifungal activity. Minimum inhibitory concentration (MIC) values were determined using standard disc diffusion method [33] (Bauer et al., 1966). The antifungal screening data revealed that, except compound **a16** all the compounds showed antifungal activities against the tested fungal strain and are more potent than Ampoterecin B and Fluconazole. From the antifungal activity data (**Table 1**), it is evident that, compounds **a12**, **a14**, **a17** and **a18** were observed as most active against the tested organisms. Its MIC value against *Candida.a* was 10 µg/mL, while fluconazole was inactive. However, it was comparable with Ampoterecin B. Especially, compound **a15**, **a19** and **a20** exhibited almost similar activity against *Candida.a* (MIC 15 µg/mL). While, compounds **a11** and **a13** showed moderate activity against the tested organism. Moreover, it showed higher activity against *Candida.a* than standard drugs. Furthermore, the MIC values of potent compounds **a12**, **a14**, **a17, a18** and **a20** (**Table 1**) showing their superior activity against bacterial and fungal strains than respective standard drugs Chloramphenicol, Pencillin, Ampoterecin B and Fluconazole. Finally, these compounds represent new structure that could be further optimized for future development of more potent and selective antimicrobial agents.

2.1.2. Anti-inflammatory activity

In the acute oral toxicity study, no mortality was noticed within 48 hours by any of the compounds up to 1500 mg/kg, p.o. dose level in the present study. However, some behavioral changes were noticed depending at the higher dose (400 mg/kg). The effect of the tested compounds as well as reference standard were measured before and 1, 2, 3, 4, 5, and 6 h after carrageenan injection. Most of the tested compounds showed a reasonable inhibition of oedema size in comparison with standard drug Diclofenac Sodium. From the obtained results **(Table 2)**, it has been observed that newly prepared compounds **(a11–a20)** reveal better anti-inflammatory properties comparable to that of Diclofenac sodium which was used as a reference standard. Structure-activity relationships based on the observed results indicated that, the type of group substitution attached to the phenyl ring plays a controlling role for developing the exhibited pharmacological properties. It has been noticed that, substitution of an electron–withdrawing group, a chlorine atom on the phenyl ring with **a12**, **a14**, **a18** and **a20** seems more favorable for constructing an anti-

inflammatory active agent. The methoxy substitution on the phenyl ring led to compounds **a15 & a17** with slightly different behavior, but has shown better activity. On the other hand, comparing the activity of the compounds **a13** and **a19,** it was observed that replacing the hydrogen atom by a nitro group reduces the anti-inflammatory activity.

EXPERIMENTAL

The biological activities were carried out at Department of Microbiology and Biotechnology, Karnatak University Dharwad and Luqman College of Pharmacy, Gulbraga, Rajiv Gandhi University, India.

3.1. Pharmacological Screening Methods

3.1.1. Antimicrobial activity assay

The disc diffusion method was conducted as per the Kirby Bauers method (Bauer et al., 1966) to screen *S.aureus*, *E.coli*, *Salmonella typhi* bacteria and *Candida albicans* fungi. *In vitro* antimicrobial activity was screened by Mueller Hinton agar (MHA) which was procured from Himedia Mumbai. The MHA plates were prepared as per the manufactures instruction and poured into sterile plates and allowed to solidify 0.1 ml of inocullum suspension was spread uniformly. The different concentrations of compounds (10, 15, 20, 25 and 30 10 µg/mL) were loaded on 6mm sterile discs. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes. The bacterial plates were kept for incubation at 37ºc for 24 hours while the fungal plate was incubated at room temperature for 48 hours. At the end of incubation inhibition zones formed around the discs were measured with zone measuring scale. The minimal inhibitory concentrations (MIC) were defined as the lowest concentrations of compound that completely inhibited the growth of each strain.

3.1.2. Anti-inflammatory activity assay

Anti-inflammatory activity was evaluated by using carrageenan induced hind paw oedema method [34,35] ((Winter et al., 1962; Prism Demo Graph Pad Software, Inc.). Albino-Wistar rats strains of either sex between 150-200 g were selected for the studies. Study protocol was approved by the institutional Animal Ethics Committee (IAEC, Reg.No. 346/ CPCSEA: Dated. 03-01-2001, Department of Pharmacy, Luqman College of pharmacy and Research, Gulbarga) before experiment. The animals were kept on diet and allowed food and water *ad libitum*. They were housed in polypropylene cages maintained under standard condition (12 h light/12 h. dark cycles at 25 ± 3 ^{o}C temperature, 35-60 % humidity). The rats were divided into fourteen groups of six rats each as described in Table 2. The control group received Tween-80 (1%) 10ml/kg p.o. The test groups received 400mg/kg p.o. of synthetic compounds. Diclofenac sodium 200 mg/kg p.o. served as standard. All the suspensions were administrated 30 min before the injection of carrageenan (0.1 ml of 1 %). The paw oedema volume was measured with the help of plethysmograph by mercury displacement method at 0, 1, 2, 3, 4 & 6 hours.

Table 1. Minimum inhibition concentrations of Compounds a11–a2o using standard antibiotics for antimicrobial activity.

Compounds	Micro-organisms used for Antimicrobial Activity (MIC values µg/mL)			
	S.aureus	***E.coli***	***Salmonella.typhi***	***Candida.a***
a11	30	>30	>30	25
a12	10	10	15	10
a13	10	20	20	25
a14	10	15	15	10
a15	>30	>30	>30	15
a16	25	15	>30	>30
a17	15	15	15	10
a18	10	10	15	10
a19	10	20	>30	20
a20	10	10	15	15
Chloramphenicol	15	15	20	
Pencillin	25	20	25	
Ampoterecin B				25
Fluconazole				25

Table 2. Anti-inflammatory activity of synthesized compounds (a11–a2o) by carrageenan-induced hind paws oedema method

Groups	Dose mg/kg p.o	Paw volume(mean ± SEM) mm					
		0hr	**1hr**	**2hr**	**3hr**	**4hr**	**6hr**
Control	Tween 80	1.256± 0.001	1.294± 0.001	1.325± 0.001	1.386± 0.001	1.443± 0.002	1.480± 0.001
Std Drug	200mg/kg	0.857±	0.665±	0.647±	0.581±	0.630±	0.768±

Diclofenac sodium		0.002	0.002	0.001	0.001	0.002	0.001
a11	400mg/kg	1.255± 0.001	1.293± 0.001	1.328± 0.001	1.387± 0.001	1.442± 0.002	1.485± 0.001
a12	400mg/kg	0.851± 0.003***	0.793± 0.002**	0.628± 0.004***	0.687± 0.001**	0.642± 0.005**	0.685± 0.002**
a13	400mg/kg	0.656± 0.001	0.593± 0.001	0.328± 0.001	0.458± 0.001	0.642± 0.002	0.544± 0.001
a14	400mg/kg	0.851± 0.003***	0.773± 0.004**	0.628± 0.002***	0.885± 0.001**	0.760± 0.005**	0.983± 0.003**
a15	400mg/kg	0.758± 0.004**	0.886± 0.005**	0.792± 0.003**	0.678± 0.001**	0.841± 0.005**	0.788± 0.006**
a16	400mg/kg	1.151± 0.002	1.123± 0.001	1.148± 0.003	1.237± 0.001	1.242± 0.004	1.185± 0.001
a17	400mg/kg	0.751± 0.004**	0.893± 0.005**	0.778± 0.003**	0.682± 0.001**	0.830± 0.005**	0.785± 0.006**
a18	400mg/kg	0.848± 0.004***	0.794± 0.005**	0.825± 0.003**	0.687± 0.002**	0.442± 0.001**	0.785± 0.004***
a19	400mg/kg	1.224± 0.002	1.210± 0.003	1.203± 0.001	1.205± 0.001	1.208± 0.004	1.211± 0.004
a20	400mg/kg	0.658± 0.004**	0.892± 0.005**	0.721± 0.002**	0.725± 0.002**	0.842± 0.001**	0.679± 0.003***

[a]Data was analyzed by unpaired one-way ANOVA test
[b]Each value represents the mean ± SEM (n = 6)
*P < 0.05, **p < 0.01, ***p < 0.001 as compared with control Student's't' test

CONCLUSION

In present study Schiff's base derivatives were screened for *in-vitro* antimicrobial and in-*vivo* anti-inflammatory activities. The compounds showed promising antimicrobial activity against all the selected human bacterial and fungal pathogens. The antibacterial activity of compounds **a12 and a20** with MIC–10 µg/mL against *S. aureus, E. coli* and 15µg/mL against *Salmonella typhi* was significantly greater than that of the reference antibiotics Chloramphenicol and penicillin. The antifungal screening results indicate that compounds **a12**, **a14**, **a17** and **a18** were the most active ones. Compounds **a12**, **a14**, **a15, a17, a18 and a20** were found to be the most potent anti-inflammatory agents.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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