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In vitro anthelmintic and antimicrobial activity of novel series of quinoxaline- 2, 3-dione -6-sulphonyl Benzimidazole (s)

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ABSTRACT

The anthelmintic activity of various novel 2, 3-dioxo-1, 2, 3, 4-tetrahydro quinoxaline-6-sulfonyl benz imidazole (s) performed on adult earthworm (*Pheretima Posthuma*). The observation was made for the time taken to paralysis and death of individual worms up to four hours of the test period. Paralysis was said to occur when the worms did not revive even in a vehicle. Death was concluded when the worms lost their motility followed with fading away of their body color. The selected newly synthesized 2, 3-dioxo-1, 2, 3, 4-tetrahydro quinoxaline-6-sulfonyl substituted benzimidazole (s) compounds were screened for antibacterial activity against two gram positive bacterial Species (*Staphylococcus aureus, Bacillus subtilis*), two Gram-negative bacterial species (*Escherichia coli and Pseudomonas aeruginosa*) and two fungi (*Aspergillus Niger and Candida Albicans*) by the cup plate method. The various five newly synthesized quinoxaline- 2, 3-Dione -6-sulphonyl benzimidazole (s) screened for in-vitro anthelmintic activity and antimicrobial Activity. It was found that quinoxaline- 2, 3-dione -6-sulphonyl benzimidazole (s) have pronounced effect as compared to plain quinoxaline -2,3-dione against worms, the gram positive, gram negative bacteria and Fungi. Among all the compounds, 2, 3-dioxo -1, 2, 3, 4-tetrahydroquinoxaline-6-sulphonyl (2-acetyl) benzimidazole have been exhibited good activity in the dose of 20 mg/ml against earthworm, bacteria and fungi like standard drug. This may be due to presence acetyl moiety in the benzimidazole ring conjugated with quinoxaline-2, 3- dione pharmacophore group. It was found that quinoxaline-2, 3-dione -6-sulphonyl benzimidazole (s) have pronounced anthelmintic and antimicrobial activity.

KEY WORDS: quinoxaline, imidazoles, *in vitro.*

1. INTRODUCTION

Among the various classes of nitrogen-containing heterocyclic compounds, quinoxalines display a broad spectrum of biological activity. They are part of various antibiotics such as Echinomycin, Levomycin, and Actinoleucin, which are known to inhibit the growth of gram-positive bacteria and also active against various transplantable tumors (Mahmoud, 2012). They are known to possess other biological potentials such as anthelmintic (Fisher, 1977), antimicrobial (Gauri Gupta and Preeti Verma, 2014), adenosine receptor antagonist, anticancer, antidepressant, anti-inflammatory, and antitubercular activity (Selvaraj Jubie, 2012; Mustafa, 2011). Quinoxaline and its mode of action that prevents DNA-directed RNA synthesis by virtue of binding to cog site on DNA (Ramalingam, 2010). Interestingly, some 2-sulphonylquinoxalines and 3- [(alkyl thio) methyl] quinoxaline 1-oxide derivatives were reported to be endowed with antibacterial and antifungal activities (Antonio Carta, 2002).

The benzimidazole ring system is an important pharmacophore in medicinal chemistry and modern drug discovery. The compound bearing benzimidazole nucleus has been of great interest to synthetic and medicinal chemists from a long time due to their unique chemical and biological properties mainly related to traditional anthelmintic like Albendazole and Oxibendazole (Manish, 2012; Srikanth Lingala, 2011). It was found that benzimidazole derivatives displayed antimicrobial, antiviral, antiproliferative agent, antitubercular, antiulcer, antioxidant, analgesic, HIV-RT inhibitor, central nervous system depressant, anticancer, DNA topoisomerase inhibitors, antibacterial and antifungal activities (Akbar, 2010).

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths is restricted to tropical regions and cause an enormous hazard to health and contribute to the prevalence of undernourishment, anemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting the principal population in endemic areas (Tagbota, 2001). The gastrointestinal helminths become resistant to currently available anthelmintic drugs.. Because the existing anthelmintics may show serious side effects in hosts such as epigastric pain, diarrhea, nausea, vomiting, headache, dizziness, edema, rashes and urticaria and are contraindicated in pregnant and lactating woman because of low safety profile. Therefore, it is the need of the hour to synthesize some novel anthelmintic agents to overcome resistance and side effects for safe and effective treatment of anthelmintic infections (Rajeev Kharb, 2012).

The number of life-threatening infectious diseases caused by multidrug-resistant bacteria have reached an alarming level in many countries around the world. Infectious diseases caused by bacteria have increased

dramatically in recent years. In spite of many significant advances in antibacterial therapy, the widespread use and misuse of antibiotics have caused the emergence of bacterial resistance to antibiotics, which is a serious threat to public health. In particular, the emergence of multidrug-resistant gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin - resistant *S. Aureus* (VRSA), and vancomycin-resistant *Enterococci* (VRE) has become a serious problem in the treatment of bacterial diseases (Deniz, 2008). Despite the great effort from the pharmaceutical industry to manage the resistance problem, the discovery and development of new mechanistic classes of antibiotics have found very little success. Therefore; the development of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today (Desai, 2013; Sudheer Babu and Selvakumar, 2013).

Hence, there is an increasing demand towards natural anthelmintic hence the synthesis of new heterocyclic's derivatives is being reported continuously and many research articles have been published specifying a wide variety of pharmacological activities. We targeted the *In vitro* biological evaluation of an anthelmintic and antimicrobial activity of quinoxaline- 2, 3-dione -6-sulphonyl benzimidazole (s) and also to study the effect of incorporation of benzimidazole with the quinoxaline-2,3-dione through the sulphonyl moiety.

2. MATERIALS AND METHODS

Procurement of synthetic derivatives for the pharmacological screening of the compounds were prepared and characterized by the reported procedure of our previously published work (Selvaraj Jubie, 2011). The structure of the tested compounds for the anthelmintic and antimicrobial activity are as follows (Fig. 1-7).

Structures of the Titled compounds

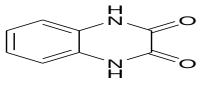

Fig.1: Quinoxaline-2,3(1H,4H)-dione (QD)

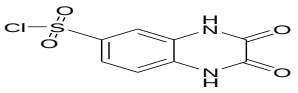

Fig.2:1,2,3,4-tetrahydro-2,3-dioxoquinoxaline-6-sulfonyl chloride (QDSC)

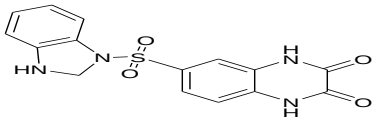

Fig.3: 1,2,3,4-tetrahydro-2,3-dioxoquinoxaline-6-sulfonyl Benzimidazole (QDSB)

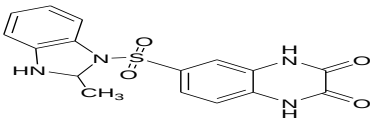

Fig. 4: 1,2,3,4-tetrahydro-2,3-dioxoquinoxaline-6-sulfonyl(2-methyl) Benzimidazole (QDS MB)

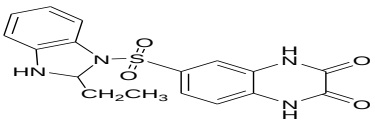

Fig .5: 1,2,3,4-tetrahydro-2,3-dioxoquinoxaline-6-sulfonyl (2-ethyl) Benzimidazole (QDSEB)

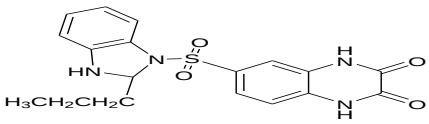

Fig . 6: 1,2,3,4-tetrahydro-2,3-dioxoquinoxaline-6-sulfonyl (2-propyl) Benzimidazole (QDSPB)

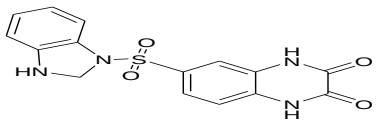

Fig .7:1,2,3,4-tetrahydro-2,3-dioxoquinoxaline-6-sulfonyl (2-acetyl) Benzimidazole (QDSAB)

2.1. Anthelmintic activity: The anthelmintic activity of selected newly synthesized 2, 3-dioxo-1, 2, 3, 4-tetrahydro quinoxaline-6-sulfonyl benzimidazole (s) were performed on adult earthworm (*Pheretima Posthuma*). Owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings, easy availability and earthworm has been used extensively for preliminary in vitro evaluation of anthelmintic compounds according to the method of Dash (2003); Munne (2012); Panda (2011). The drug is water insoluble, hence a suspension of the drug was prepared in which a 0.05% of carboxymethyl cellulose was used as a suspending agent for making the concentration of 10, 20 mg/ml of QD, Poured 10 ml of each suspension in a separate petri dish. Left six worms in each Petri dish.The observation was made for the time taken to paralysis and death of individual worms up to 4 h of the test period. Paralysis was said to occur when the worms did not revive even in the vehicle. Death was concluded when the worms lost their motility followed with fading away of their body color.

2.2. Antimicrobial activity: The synthesized 2, 3-dioxo-1, 2, 3, 4-tetrahydro quinoxaline-6-sulfonyl benzimidazole (s) compounds were screened for antibacterial activity against two gram positive bacterial Species (*Staphylococcus aureus (SA), Bacillus subtilis(BC)*), two Gram-negative bacterial species (*Escherichia coli (EC) and Pseudomonas aeruginosa(PA)*) and two fungi (*Aspergillus Niger(AN) and Candida Albicans(CA)*) by the cup plate method (Dharmchand Prasad Singh, 2010). The sterilized nutrient agar medium was distributed 100 ml each

in two 250 ml conical flasks and allowed to cool to room temperature. To these media, 18-24 h grown bacteria & fungi sub-cultures were added and shaken thoroughly to ensure uniform distribution of organism's throughout the medium. This agar medium was distributed in equal portions, in sterilized Petri dishes, ensuring that each Petri dish contains about 45-50 ml of the medium. The medium was allowed for solidification. The cups were made with the help of a sterile cork borer (6 mm diameter) punching into the set of agar media. The solutions of required concentrations (20 mg/ml) of test compounds were prepared by dissolving the compounds in carboxymethyl cellulose were filled into the cups with 1ml of the respective solution. Then, the Petri dishes were kept for incubation in an inverted position for 24-48 h at 37^{o}C in an incubator. When growth inhibition zones were developed surrounding each cup, their diameter in mm was measured and compared with that of the standard drugs Ofloxacin, Griseofulvin.

2.3. Statistical Analysis: Results were expressed as mean±s.e.m. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test, with the level of significance at $P < 0.05$.

3. RESULTS AND DISCUSSION

From the data shown in Table 1, title compounds acquired the anthelmintic activity. A closer inspection of data from tables indicates that 2,3-dioxo -1,2,3,4-tetrahydroquinoxaline-6-sulphonyl (2-acetyl) benzimidazole and 2, 3-dioxo -1, 2, 3, 4- tetrahydroquinoxaline-6-sulphonyl (2 methyl) benzimidazole showed better paralytic activity. They showed potential anthelmintic activity with respect to the death of worms. All the compounds have shown potential anthelmintic activity but less than the standard drug. Quinoxaline-2,3-dione and 2, 3-dioxo -1, 2, 3, 4-tetrahydroquinoxaline-6- sulphonyl chloride showed less activity than the title compounds. The compound 2, 3-dioxo -1, 2, 3, 4- tetrahydroquinoxaline-6-sulphonyl benzimidazole showed significant activity.

From the data shown in Table 2, it is clear that; the title compounds showed good antimicrobial activity. Ofloxacin and Griseofulvin are used as the standard. Quinoxaline -2, 3-dione compound is generally devoid of activity towards the tested gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*). 2, 3-dioxo -1, 2, 3, 4-tetrahydroquinoxaline-6- sulphonyl chloride showed moderate antibacterial activity and least antifungal activity. 2, 3-dioxo -1, 2, 3, 4- tetrahydroquinoxaline-6-sulphonyl benzimidazole exhibited significant antibacterial and antifungal activity. 2, 3-dioxo -1, 2, 3, 4- tetrahydroquinoxaline-6-sulphonyl (2 methyl) benzimidazole and 2, 3-dioxo -1, 2, 3, 4- tetrahydroquinoxaline-6-sulphonyl (2 acetyl) benzimidazole exhibited good antimicrobial activity compared to standard drugs.

Discussion: Helminthiasis, the condition resulting from worm infestation, is one of the major prevalent diseases in the world, particularly in the tropical countries. Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for the widespread nature of this disease in the developing countries including India. Helminthiasis is prevalent globally one-third of the world's population harbors them but is more common in developing countries with poor personal and environmental hygiene (Walter, 1985).

All the titled compounds were tested for anthelmintic activity against adult earthworms (*P. posthuma*) due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Piperazine citrate, one of the reference compounds in the present study is effective in a broad range of helminthic infections, including roundworms, hookworms, whipworms, pinworms and is a cheap and readily available anthelmintic agent with very wide therapeutic index (Onuaguluchi, 1966). All compounds showed their activity at a minimal dose of 10 mg/ml. While increasing the concentration of the compounds (20 mg/ml) significantly ($P<0.05$) lowered the time taken for paralysis and death of the selected worms. From the observations made, the higher concentration of compounds produced a paralytic effect much earlier and the time to death was shorter for the worms. Quinoxaline -2, 3-dione is generally devoid of activity against gram-negative bacteria and fungi, but little activity against gram-positive bacteria. 2, 3-dioxo -1, 2, 3, 4- tetrahydroquinoxaline-6-sulphonylchloride have lesser antimicrobial activity. But the substitution of benzimidazoles in 2, 3-dioxo -1, 2, 3, 4- tetrahydro quinoxaline-6-sulphonylchloride are active towards the tested gram-positive, gram-negative bacteria (*Bacillus subtilis, Staphylococcus aureus*) and fungi (*Candida Albicans, Aspergillus niger*). It was found that 2, 3-dioxo-1, 2, 3, 4-tetrahydroquinoxaline-6-sulfonyl benzimidazole (s) have pronounced effect as compared to the precursor compound quinoxaline -2,3-dione against worms, the gram positive, gram negative bacteria and fungi. Among all the compounds, 2,3-dioxo -1,2,3,4-tetrahydroquinoxaline-6-sulphonyl (2-acetyl)benzimidazole having the better activity than the other compounds. This effect is because of the presence of acetyl moiety which means that electron withdrawing group is essential for anthelmintic activity in benzimidazole ring in the same molecular structural frame. So they were observed with significant antimicrobial activity against *S. aureus* which may be a new light for the treatment of S. aureus. Among various pathogenic bacterial strains, E. coli is the leading organism responsible for urinary tract infections. This study also revealed that the effect of incorporation of quinoxaline - 2, 3 -Dione with benzimidazole (2, 3-dioxo -1, 2, 3, 4- tetrahydroquinoxaline-6-sulphonylbenzimidazole) have shown better anthelmintic, antibacterial and antifungal activity to a certain extent.

Table.1.Anthelmintic activity of synthesized compounds

Compounds	Concentration mg/ml	Time taken for Paralysis in min.(Mean & SEM)	Time taken for Death in min.(Mean & SEM)
Control (vehicle)	--	--	--
Standard Piperazine citrate	20	06.36±0.26	10.09±0.02
QD	10	18.90±0.21	22.01±0.15
	20	16.65±0.12	20.07±0.25
QDSC	10	19.20±0.11	22.04±0.33
	20	17.97±0.87	21.40± 0.17
QDSB	10	15.92±0.23	18.33±0.17
	20	12.62±0.13	15.67±0.34
QDSMB	10	13.81± 0.22	14.90±0.19
	20	10.80±0.12	13.21±0.15
QDSAB	10	10.23±0.10	13.77±0.33
	20	08.12±0.18	11.90±0.15

P value was calculated by comparing with control by one-way ANOVA. Control worms were alive up to 24h of observation. P< 0.05.

Table.2.Antimicrobial activity of synthesized compounds

	Zone of Inhibition					
Compounds in 20mg/ml	**Antibacterial Activity**				**Antifungal Activity**	
	Gram positive bacteria		**Gram negative active**		**Fungi**	
Control	--	--	--	--	--	--
Standard Ofloxacin	++	++	++	++	--	--
Griseofulvin	--	--	--	--	++	++
QD	+	+	--	--	--	--
QDSC	++	++	+	+	+	+
QDSB	++	++	++	+	++	++
QDSMB	++	++	++	++	+++	+++
QDSAB	+++	+++	+++	+++	+++	+++

The data represent zones of inhibition (mm) as follows: - 0 mm, + 1-10 mm, ++ 10-15 mm, +++15-30 mm.

4. CONCLUSION

The present study indicated that synthesized quinoxaline -2,3- dione derivatives showed better activity than its precursor compound 1 and the substitution of sulphonyl benzimidazole group in the quinoxaline 2,3dione derivatives vary the activity. Quinoxaline-2,3-dione having sulphonyl benzimidazole moieties class of compounds certainly holds great promise towards the good anthelmintic and antimicrobial activity due to conjugate with pharmacophore group such as benzimidazole ring in the same molecular structural frame. However, further studies for the In-Vivo evaluation of antimicrobial and anthelmintic activities and molecular studies of Quinoxaline- 2, 3-dione -6-sulphonyl Benzimidazole (s) compounds are warranted.

REFERENCES

Akbar M, Naser F, Mehdi K, Mansourah M, Synthesis and antifungal activity of novel 2-benzimidazolylimino-5-arylidene thiazolidinones, J.Heterocycl.Chem., 47, 2010, 77-80.

Antonio Carta, Giuseppe Paglietti, Mohammad E, Rahbar Nikookar, Paolo Sanna, Leonardo Sechi, Stefania Zanetti, Novel substituted quinoxaline 1, 4-dioxides with in vitro antimycobacterial and anti-candida activity, Eur.J.Med.Chem., 37, 2002, 355–66.

Dash GK, Mishra B, Panda A, Patro CP, Ganapaty S, Indian J.Nat.Products, 19(3), 2003, 24-6.

Deniz SD, Tijen Onkol, Semiha Ozkan, Selda Ozgen, Fethi Sahin, Synthesis and Antimicrobial Activity of Some 3(2H)-Pyridazinone and 1(2H)- Phthalazinone Derivatives, Turk.J.Chem., 32, 2008, 469-79.

Desai NC, Amit Dodiya, Niraj Shihory. Synthesis and antimicrobial activity of novel quinazolinone–thiazolidine–quinoline compounds, J.Saudi.Chem.Soc., 17, 2013, 259–67.

Dharmchand Prasad Singh, Sanjay Kumar Deivedi, Syed Riaz Hashim, Ram Gopal Singhal, Synthesis and Antimicrobial Activity of Some New Quinoxaline Derivatives, Pharmaceuticals, 3, 2010, 2416-25.

Fisher MH, Lusi A, Eqerton JR, Anthelmintic dihydroquinoxalino [2,3-b] Quinoxalines, Pharm.Sci., 66, 1977, 1349-52.

Gauri Gupta, Preeti Verma, Antimicrobial Activity of Quinoxaline Derivatives, Chem.Sci.Trans., 3, 2014, 876-884.

Indian Pharmacopoeia, Vol II, 1996, A-100-8.

Mahmoud AA, Mohamed MY, Use of Modern Technique for Synthesis of Quinoxaline Derivatives as Potential Anti-Virus Compounds, Der.Pharma.Chemica, 4, 2012, 1323-29.

Manish BS, Vilasrao JK, Deepali MJ, Priyanka SG, Priyanka LG, Synthesis and Evaluation of Novel Anthelmentic Benzimidazole Derivatives, JRPC, 2, 2012, 132-6.

Munne SL, Parwate DV, Ingle VN, In-Vitro Anthelmintic Activity of Citrus Sinensis Seed Coats, IJPR,2, 2012, 83-5

Mustafa AH, Design Synthesis and Antimicrobial Activity of New 1,4- disubstituted Octahydroquinoxaline-2, 3-dione derivatives, Bull Korean Chem.Soc., 32, 2011, 1511-18.

Onuaguluchi G, Some aspects of the pharmacology of piperazine citrate and the antiascaris fraction of the ethanolic extract of the bark of ERIN tree (Polyadoa umbellate- Dalziel), West Afri.Med.J., 15, 1966, 22-5.

Panda SK, Das D, Tripathy NK, Evaluation of Anthelmintic activity of Chlorophytum borivilianum santapau & fernandes, IJRBS, 2, 2011, 676-79.

Rajeev Kharb, Prabodh Chander Sharma, Anil Bhandari, Mohammad Shahar Ya, Synthesis, spectral characterization and anthelmintic evaluation of some novel imidazole bearing triazole derivatives, Der. Pharmacia Lettre, 4 (2), 2012, 652-7.

Ramalingam P, Ganapaty S, Babu Rao Ch. In-vitro antitubercular and antimicrobial activities of 1- substituted quinoxaline-2, 3(1H, 4H) – diones, Bio org.Med.Chem.Lett., 20, 2010, 406-8.

Selvaraj Jubie, Rajamanickam Gayathri, Ammayappan Rajam Srividya, Rajagopal Kalirajan, Prabakaran Prabitha, Sundaram Sankar, Kannan Elango, Synthesis and Characterization of some Novel Quinoxaline-2, 3-Dione Derivatives: A Preliminary Investigation on their Activity against a Human Epithelial Carcinoma Cell Line, Lett.Drug Des.Discov., 8, 2011, 317-20.

Selvaraj Jubie, Rajamanickam Gayathri, Rajagopal Kalirajan. Synthesis and Neuropharmacological Evaluation of Some Novel Quinoxaline 2, 3-Dione Derivatives, Scientific World J, 718023, 2012, 1-8.

Srikanth Lingala, Raghunandan Nerella, Samba Siva Rao KRS, Synthesis, antimicrobial and anthelmintic activity of some novel benzimidazole derivatives, Der.Pharma.Chemica, 3, 2011, 344-52.

Sudheer Babu I, Selvakumar S, In-Vitro Antimicrobial and In-Vivo Central Nervous System - Locomotor Activities of Some Synthesized 2- [(1-((Phenyl Amino) Methyl) Substituted 1- Benzoimidazol-2-Yl) Alkyl] Isoindoline-1, 3-Diones, IJBPR, 4, 2013, 1123-30.

Tagbota S, Townson S, Antiparasitic properties of medicinal and other naturally occurring products, Adv. Parasitol., 50, 2001, 199-205.

Walter PJ, Richard KK. In: W.C. Campbell and Rew LS, Chemotherapy of parasitic infections, Plenum, New York: 1985, 278-539.