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Evaluation of anti-inflammatory and antibacterial potential of newly synthesized 4-(2-Keto-1-benzimidazollinyl) derivatives of piperidine

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Abstract: Benzimidazole and its derivatives found variety of biological activities, for the searching of its potent anti-inflammatory analogues, we synthesized four novel 4-(2-keto-1-benzimidazollinyl) piperidine derivatives (Q1 to Q4) by refluxing piperidine with substituted imidazole and subjected to *in-vitro* anti-inflammatory (ROS, NO) and antibacterial activities, structures were elucidated using spectroscopic techniques. Results revealed that compound Q1 showed most effective anti-inflammatory activity with IC_{50} 7.6±1.3 µg/ml compared with standard Ibuprofen having IC_{50} 11.2±1.9µg/mL. Compound Q3 showed good activity for Nitrite accumulation by stimulating macrophages test similar to standard N_G Methyl L-arginine acetate with IC_{50} value 24.2±0.8µg/mL. The antibacterial activity of these compounds were evaluated against selected Gram+ve *E. faecalis, C. diphtheriae, S. aureus* and Gram -ve organism *E. coli, Enterobacter aerogenes* and *P. aeruginosa*. Synthesized compounds showed low to moderate level of antibacterial activity Q1 showed the highest antibacterial activity against *Enterococcus faecalis* and *Escherichia coli* with zone of inhibition 18mm and Q3 showed highest activity against *Corynebacterium diptheriae* (ZOI:18mm). Structure-activity relationship (SAR) study revealed that among all the synthesized compounds unsubstituted naphthalene (Q1) and phenyl (Q3) ring containing derivatives were most potent.

Keywords: Synthesis, 4-(2-keto-1-benzimidazollinyl) piperidine, anti-inflammatory, antibacterial, reactive oxygen species (ROS), Nitric oxide (NO).

INTRODUCTION

The tricyclic Benzimidazolinyl piperidine is heterocyclic aromatic compound formed by the fusion of benzene, imidazolinone and piperidine nucleus. Benzimidazolinyl piperidines are found to be a potential target for synthesis of several compounds having potential biological activities (Bender *et al.,* 1996; David *et al.,* 2008; Patel *et al*., 2014; Ekta *et al.*, 2018). Recently active antileishmanial alkyl benzimidazoles were synthesized (Michele *et al*., 2017). Is has been found that benzimidazoles are used for the treatment of urinary tract infections (El-Gohary *et al*., 2017). Bender has reported significant antifungal and antibacterial analogues (Bender *et al*., 1996). It has been found by research that many antifungal and antibacterial analogues have also profound antimicrobial activity containing substituted alkyl and aryl groups. Nitroimidazoles containing compounds has been synthesized having antiamoebic properties (Vanelle *et al*., 2000; Kazimierczuk *et al.,* 2002). Benzimidazole optimisation results wide range of therapeutically active antacids (Keri *et al.,* 2015; Mamedov, 2016). Immunomodulatory active compounds and drugs have progressively being produced for the treatment of various human diseases including inflammatory disorders. The process of inflammation causes annihilation of tissues (Shah *et al*., 2012; Kathy *et al.,* 2017). Reactive oxygen species (ROS) are causes of many sevior pathogenesis including tumor, atherosclerosis, diabetes, myocardial localized necrosis and Alzheimer's illness (Demirkiran *et al.*, 2009). Under normal biological and physiological circumstances Nitric oxide gives anti-inflammatory effects, as Nitric oxide proved to be a pro-inflammatory mediator that induces inflammation Inhibitor of NO while arginine synthesized analogues have been used for the treatment of inflammation that induced by NO (Sharma *et al.*, 2007).

MATERIALS AND METHODS

Chemicals were procured from Sigma Aldrich (Steinheim, Germany) and Merck (Hohenbrunn, Germany). The analytical grade solvents were used for analysis. Pre-coated silica gel TLC plates (GF-254) were used for analyzing the spot under UV254 lamp (Toshiba, Japan). For IR spectral analysis Jasco-320-A spectro-

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photometer (Easton, MD 21601 USA) was used. Bruker spectrophotometer (Karlsruhe, Germany) was used for ^{1}H-NMR spectra and obtained at 300,400 and 500 MHz in DMSO-d_6 and MeOD. Mass spectra were recorded using JEOL mass spectrometer (Tokyo, Japan).

Synthesis and spectral characterization

Synthesis of 4(1-benzimidazolinyl-2-Keto)-Piperidine was done by refluxing equimolar with 2-acetophenone-2-bromide, 4` nitroacetophenone-2-bromide, 3-phenoxy propyl bromide and 2,4 dibromo acetophenone separately in a round bottom flask by dissolved in 15-25mL methanol upon magnetic stirring (2 to 3 hours/25°C) (Scheme 1). Completion of the reaction was analyzed by taking thin layer chromatography. Crude solid part of the products first filtered and purified by washing with several portions of acetone.

1 - (2- (naphthalen-1-yl)-ethyl-2oxo) - 4 - (2,3-dihydro-2oxo-benzimidazol1yl) piperidiniumbromide (Q1)

Yields: 85%: White shiny crystals; mp: 217±0.3; Solubility: DMSO, Ethanol; $UV_{\lambda max}$ (MeOH) nm: 234, 238; HR-EIMS: m/z (%) 465 ($C_{24}H_{24}N_3O_2Br$ calcd 465.11); ^{1}H-NMR (300 MHz:DMSO) δm (ppm) 2.482-2.499 (s, 2H, H-3, H-5); 3.158-3.557 (m, 6H, H-2, H-6, H-4); 3.875(s, 1H, H-1'"); 7.123-7.145 (d, 2H, H-6", *J*= 8.8 Hz); 7.759-7.780 (d,2H, H-6',J=8.4Hz); 7.817-7.843 (m, 4H, H-3', H-7', H-5"); 7.884-7.889 (s, 2H, H-7"); 7.988-8.010 (d, 2H, H-4', H-8'); (IR:KBr) cm^{-1}: 750.14 (*o*-substitution), 1319.31 (C-N), 1516.05 (C=C), 1639.16 (C=C aromatic), 1716 (C=O), 28.23.40 (Ar-H), 3065.97 (N-H)

1- (2- (4-nitrophenyl) - 2-oxoethyl) - 4 - (2,3-dihydro-2oxo-benzimidazol1yl) piperidiniumbromide (Q2)

Yield: 88% ; mp: 209±0.6; Solubility: DMSO, Ethanol, Methanol; $UV_{\Lambda max}$ (MeOH) nm: 220, 238, 251; HR-EIMS: m/z (%) 461 ($C_{20}H_{21}BrN_4O_4$ caltd. 461.3091); ^{1}H-NMR (MeOD: 500 mHz) δ (ppm): 2.032-2.060 (d, 2H, *J*-15 Hz, H-3, H-5); 3.155-3.173 (m, 2H, H-2, H-6); 3.303 (S, 2H, H-4); 4.498-4.547(M, 2H, H-7); 7.070- 7.113 (m, 3H, H-7", H-6",H-5"); 7.254-7.271 (m 6H, H-2', H-3', H-5', H-6', H-4"); (IR:KBr) cm^{-1}): 748.12 (o-substitution), 1369.65 (C-N), 1415 (Ar-NO_2), 1649.04 (C=C aromatic), 1716 (C=O), 2831.12 (Ar-H), 3065.97 (N-H).

Mono (2-oxo-1-(1-(3-phenoxypropyl) piperidinium - 4 - (2,3-dihydro-2oxo-benzimidazol1yl monobromide (Q3)

Yields: 83.5% : White crystals; mp: 209±0.7; Solubilty: DMSO, Ethanol, Methanol; $UV_{\lambda max}$ (MeOH) nm: 233; HR-EIMS : m/z (%) 416 ($C_{21}H_{26}BrN_3O$ caltd 416.3546); ^{1}H-NMR(MeOH, 300 MHz) δm (ppm); 2.05 (m,4H,H-3, H-5); 3.17-3.27(m, 6H, H-1, H-2); 3791-3.799 (s, 1H, H-1'"); 7.25(m, 1H, H-6"); 7.601-7.698 (d, 2H, H-3', H-5', H-5", H-6", *J*=29.1 Hz); 8.090-8.1301 (d, 2H, H-2', H-6', J=12 Hz); 8.4971 (m, 1H, H-4"); (IR:KBr) cm^{-1}: 742.59 (*o*-substitution); 1192.01, (*Ar*-O-CH2), 1371.39 (C-N), 1566.20 (C=C), 1645.28 (C=C aromatic), 1718 (C=O), 2829.57 (*Ar*-H), 3066.82 (N-H)

1-(2-(4-bromophenyl)-2-oxoethyl) - 4 - (2,3-dihydro-2oxo-benzimidazol1yl) piperidinium bromide (Q4)

Yield: 90.5%; white shiny crystals; mp: 218±0.3˚C; Solubility: DMSO, Methanol; $UV_{\lambda max}$ (MeOH) nm: 226, 240; HR-EIMS: m/z (%) 495($C_{20}H_{21}Br_2N_3O_2$ Caltd 495.22);^{1}H-NMR (MeOD, 500 MHz) δm (ppm); 2.021-2.049 (s,1H, H-3, H-5); 2.0592-2.718 (m, 6H, H-2, H-4, H-6) ; 3.152-3.210 (t, 4H, H-1'", J=29Hz); 7.061-7.112 (d, 4H, H-6", J=25.5 Hz); 7.691-7.709 (s, 2H, H-3', H-5'); 7.845-7.861(S, 2H, H-2', H-6'); 7.947-7.964 (s, 2H, H-5", H-7"); (IR: KBr) cm^{-1}: 740.67 (*o*-substitution), 1369.46 (C-N), 1566.29 (C=C), 1645.26 (C=C aromatic), 1718 (C=O), 2829.57 (*Ar*-H), 3066.82 (N-H)

In-vitro Anti-inflammatory Activity

Methods

Helfand *et al.* (1982) described Luminol enhanced chemiluminescense method for analysis and in this regard Ibuprofen was used as standard (Helfand, Werkmeister, & Roder, 1982). Nitric oxide production was observed given by griess method following procedure given by Amparo *et al*. (2005)

Antibacterial Activity

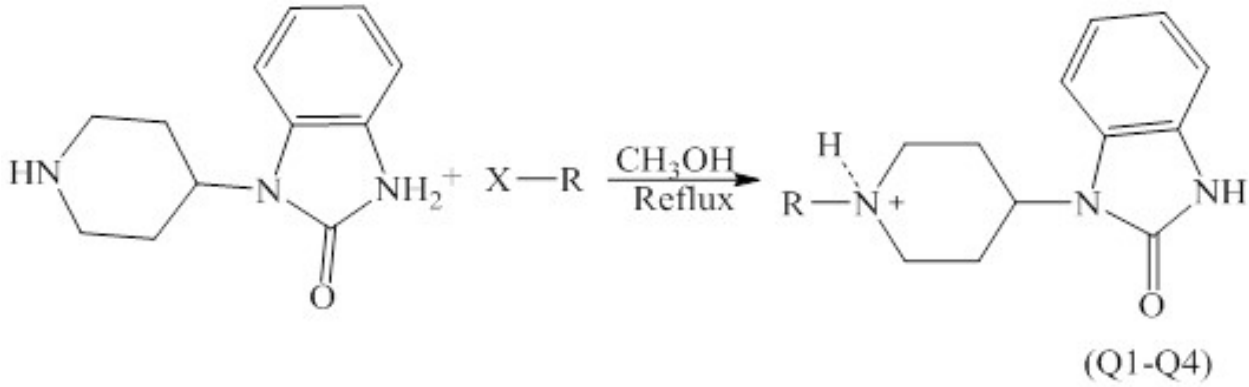
Method

Antibacterial activity of compounds was performed against Gram (+ve) organism including *Enterococcus faecalis, Corynebacterium diphtheriae, Staphylococcus aureus*, and Gram (-ve) microorganism included *Escherichia coli, Enterobacter aerogenes and Pseudomonas aeruginosa* by followed procedure given by Saleem *et al*. (2013). All samples were prepared by 25µg/mL in DMSO (Dimethyl Sulfoxide) and the further procedure was done by agar diffusion method given by Alves *et al*. (2000) and Stepanovic *et al*. (2003).

RESULTS

4-(2-keto-1-benzimidazollinyl) piperidine derivatives were successfully synthesized in quantitative yield and characterized by the spectroscopic procedure. Infrared spectra revealed that stretching of bond showed the presence of carbonyl group of ketone moiety at frequency 1716 and 1718, N-H stretching at 3066.82 and 3065.97 confirmed the presence of formation of piperidine.

All the compounds were evaluated for *in-vitro* studies including anti-inflammatory by two methods include oxidative burst (ROS) assay and Nitric oxide (NO) assay, results presented in table 1 represents % inhibitory effect of compounds and IC_{50} values of active compounds on production of reactive oxygen species (ROS) and nitric oxide (NO) mean ± SD of triplicates and all statistical analysis was performed using Excel based formula using



Scheme 1: Synthetic Scheme of 4-(2-Keto-1-benzimidazollinyl) Piperidine derivatives Where, X: Br. R: $C_{13}H_{12}O$ (Q1); $C_9H_9NO_3$(Q2), $C_{10}H_{14}O$(Q3); C_9H_9BrO(Q4)

Table 1: *In vitro* Anti-inflammatory activity (ROS, NO)

Anti-inflammatory Activity				
	ROS		NO	
Compound Code	%inhibition (25µg/mL)	IC_{50}±SD (µg/mL)	% inhibition (25µg/mL)	IC_{50}± SD (µg/mL)
Q1	80.43±1.06	7.6±1.3	25.6±3.0	-
Q2	21.3±8.6	-	18.4±2.3	-
Q3	31.14±1.16	-	91.7 ± 2.6	24.75±0.97
Q4	30.62±2.79	-	-20.5±8.5	-
Ibuprofen[1]	73.2 ± 1.4	11.2±1.9	-	-
N^GMethyl-L-arginineacetate[2]	-	-	65.6±1.1	24.2±0.8

[1] Standard for ROS: [2] Standard for NO

Table 2: Antibacterial activity against selected organisms

	Zone of Inhibition (mm)					
Compound Code	Gram positive organism			Gram negative organism		
	Ef	*Cd*	*Sa*	*Ec*	*E*	*Pa*
Q1	18	-	9	18	9	-
Q2	-	12	14	10	10	10
Q3	10	18	8	12	-	11
Q4	8	-	20	13	-	-
Amikacin	25	30	25	21	20	23

*(Gram Positive) Enterococcus *Faecalis=EF; Corynebacterium diphtheria= Cd; Staphylococcus aureus= Sa;* (Gram Negative) *Escherichia coli=Ec; Enterobacter= E; Pseudomonas aeruginosa= Pa*

Microsoft Excel 2010. These synthesized compounds was evaluated further for antibacterial studies by agar disc diffusion method using Amikacin as standard drug showed in table 2 and graphically represented in fig. 1 using MS Word 2010.

DISCUSSION

Table 1 Shown anti-inflammatory activity of four 4-(2-keto-1-benzimidazollinyl) piperidine derivatives, It has been found that compound Q1 displayed most significant inhibitory activity for phagocytes ROS (80.43%) when compared to standard (73.2%). Compound Q3 showed comparable inhibitory activity in the production of Nitric oxide (NO) with IC_{50} value 24.75µg/mL.

Antibacterial activity

Tested compounds showed low to moderate level of activity against, both, Gram-ve and Gram+ve bacteria (table 2). Compounds Q1 showed highest antibacterial activity for seveal micro-organism, against *Escherichia coli* and *Enterococcus faecalis* (ZOI: 18mm). A moderate level of antibacterial activity was exhibited by compound Q2 against *Staphylococcus aureus* (ZOI: 14mm). Compound Q3 showed the highest antibacterial activity against *Corynebacterium diptheriae* (ZOI: 18mm). Highest inhibitory activity against *Staphylococcus aureus* (ZOI: 20mm) was exhibited by compound Q4.

Structure-activity relationship (SAR)

Regarding structure activity relationship of tested compound it is very clear that for anti-inflammatory activity unsubstituted phenyl group plays very important role for activity compounds Q1 and Q3 containing naphthalene and phenyl group showed significant activity however substitution with electronegative groups such as bromo (Br) and nitro (NO_2) group decreases the activity. Along with phenyl group presence of carboxylic group is also important for oxidative burst method (fig. 2).

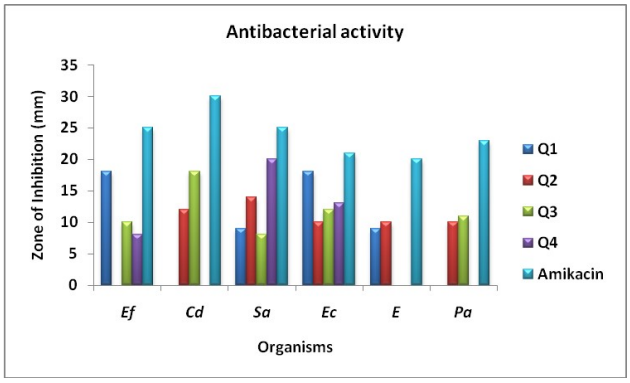


Fig. 1: Antibacterial activity of compounds (Q1-Q4) at 25µg/mL concentration.

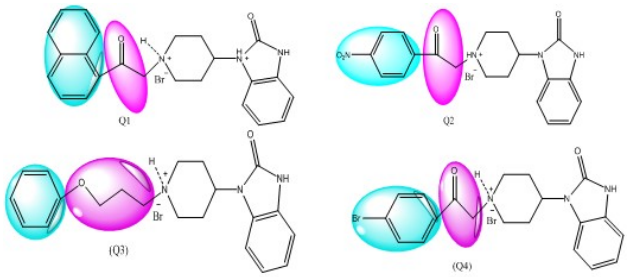


Fig. 2: Substituents presents in compounds

Compounds containing different substitution showed varied activity against selected microbes at tested dose of 25µg/mL. Compound containing bromo substituted phenyl ring (Q4) showed significant activity against *Staphylococcus aureus,* similarly compounds containing naphthalene ring showed good activity against *Enterococcus Faecalis* and *Escherichia coli.*

CONCLUSION

Conclusively, Synthesized 4-(2-Keto-1-benzimidazollinyl) Piperidine derivatives (Q1-Q4) were evaluated for their (*in vitro*) anti-inflammatory and antibacterial activities.

All the synthesized derivatives (Q1-Q4) showed varying capacity on antibacterial account. Compound Q1 showed high potential to reduce the inflammation as compared with potent anti-inflammatory agent Ibuprofen while compound Q3 have good nitric oxide inhibitory activity compared with standard N^GMethyl-L-arginineacetate. Results revealed that 4-(2-Keto-1-benzimidazollinyl) Piperidines can be further proceed as a lead in future research for anti-inflammatory agents and other biological activities.

REFERENCES

Alves T de A, Silva AF, Brandao M, Grandi TSM, Smânia E de FA, Smania Junior A and Zani CL (2000). Biological screening of Brazilian medicinal plants. *Mem. I. Oswaldo. Cruz.,* **95**(3): 367-373.

Amparo Andrade M, Siles-Lucas M, Perez Arellano JL, Pou Barreto C, Valladares B, Espinoza E and Muro A (2005). Increased rat alveolar macrophage expression of functional iNOS induced by a Dirofilaria immitis immunoglobulin superfamily protein. *Nitric Oxide*, **13**(4): 217-225.

Bender W, Hansen J, Paessens A, Wolfgang R and Raddatz SWH (1996). Substituted (2-oxo-1-benzimidazolinyl)-peperidines, process for their preparation, and use as anti-retroviral agents. US Patent No. US5571921A.

David GC, Ian Thomson Forbes, Vincenzo Garzya and Dale James Johnson PAW (2008). Compounds which have activity at m1 receptor and their uses in medicine. US Patent No.US8344000B2.

Demirkiran O, Ahmed Mesaik M, Beynek H, Abbaskhan A and Iqbal Choudhary M (2009). Cellular reactive oxygen species inhibitory constituents of Hypericum thasium Griseb. *Phytochemistry*, **70**(2): 244-249.

Ekta KMG (2018). Synthesis and biological evaluation of benzimidazole. *Int. J. Res. Pharm. Chem.,* **8**(1): 61-68.

El-Gohary NS and Shaaban MI (2017). Synthesis and biological evaluation of a new series of benzimidazole derivatives as antimicrobial, antiquorumsensing and antitumor agents. *Eur. J. Med. Chem.,* **131**: 255-262.

Helfand SL, Werkmeister J and Roder JC (1982). Chemiluminescence response of human natural killer cells. I. The relationship between target cell binding, chemiluminescence and cytolysis. *J. Exp. Med.,* **156**(2): 492-505.

Kathy KG, Rhian MT, Jay LZ, Sergey D, William C, Yeong RC, David and GH Aruni B (2017). Measurement of reactive oxygen species. *Reactive Nitrogen Species and Redox-Dependent Signaling in the Cardiovascular System,* **119**(5): e39-e75.

Kazimierczuk Z, Upcroft JA, Upcroft P, Gorska A, Starooeciak B and Laudy A (2002). Synthesis, antiprotozoal and antibacterial activity of nitro-and halogeno-substituted benzimidazole derivatives. *Acta Biochim Pol.*, **49**(1): 185-195.

Keri RS, Hiremathad A, Budagumpi S and Nagaraja BM (2015). Comprehensive review in current developments of benzimidazole based medicinal chemistry. *Chem. Biol. Drug Des.,* **86**(1): 19-65.

Mamedov VA (2016). Recent advances in the synthesis of benzimidazol (on) es via rearrangements of quinoxalin (on) es. *RSC Adv.,* **6**: 42132-42172.

Michele T, Elena G, Francesca P, Nicoletta B, Silivia P,

Bruno T, Roberta L, Fabio S and Anna S (2017). Benzimidazole derivatives endowed with potent antileishmanial activity. *J. Enzyme. Inhib. Med. Chem.,* **33**(1): 210-226.

Patel V, Bhatt N, Bhatt P and Joshi HD (2014). Synthesis and pharmacological evaluation of novel 1-(piperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one derivatives as potential antimicrobial agents. *Med. Chem. Res.,* **23**(4): 2133-2139.

Salem-Milani A, Balaei-Gajan E, Rahimi S, Moosavi Z, Abdollahi A, Zakeri-Milani P and Bolourian M (2013). Antibacterial effect of diclofenac sodium on enterococcus faecalis. *J. Dent. (Tehran).,* **10**(1): 16-22.

Shah MR, Arfan M, Amin H, Hussain Z, Qadir MI, Choudhary MI and Khan IU (2012). Synthesis of new bergenin derivatives as potent inhibitors of inflammatory mediators NO and TNF-α. Bioorg. *Med. Chem. Lett.,* **22**(8): 2744-2747.

Sharma JN, Al-Omran A, Parvathy SS (2007). Role of nitric oxide in inflammatory diseases. *Inflammo-pharmacology*, **15**(6): 252-259.

Stepanovic S, Djukic V, Djordjevic V and Djukic S (2003). Influence of the incubation atmosphere on the production of biofilm by staphylococci. *Clin. Microbiol. Infect.,* **9**(9): 955-958.

Vanelle P, Meuche J, Maldonado J, Crozet MP, Delmas F and Timon-David P (2000). Functional derivatives of 5-benzo[1,3]dioxol-5-yl-1-methyl-1H-imidazole-2-carbaldehyde and evaluation of leishmanicidal activity. *Eur. J. Med. Chem.,* **35**(1): 157-162.