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Synthesis and Biological Activity of novel 3-(1-chloropiperidin-4-yl)-6-fluoro benzisoxazole.

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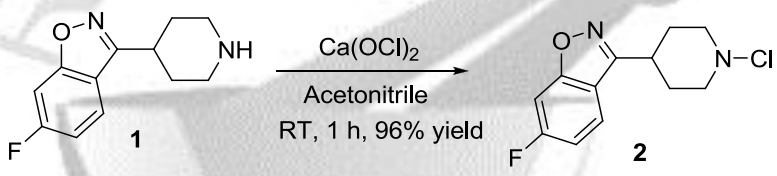
ABSTRACT

A synthèsis of novel 3-(1-chloropiperidin-4-yl)-6-fluoro benzisoxazole was carried out under mild réaction conditions using 1.2 équivalents of calcium hypochlorite. Interestingly, the developed method does not involve any additives like acids or bases and provides 96% of isolated yields at room temperature. This novel molécule, 3-(1-chloropiperidin-4-yl)-6-fluorobenzisoxazole **2** was stable at ambient conditions and stereo chemistry was established the single crystal XRD technique. Compound **2** was exhibited potent antioxidant activity (107 µg/mL), and also shown their inhibition on diabetes causing enzymes such as *alpha amylase* (270 µg/mL) and *alpha glucosidase* (37 µg/mL).



Keywords: Calcium hypochlorite, N-Chloro benzisoxazole, anti-oxidant and antidiabetic activities

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INTRODUCTION

Diabetes mellitus is a disorder characterized by increased blood glucose levels and it is associated with a host of changes in micronutrient status [1-3]. Several studies showed increased oxidative stress in both type I and type II diabetes leading to oxidative damage [4]. It is also widely reported that antioxidants intake significantly reduces the oxidative stress. Apart from oxidative stress alpha amylase and alpha glucosidase involved in the carbohydrate metabolism also play a major role in diabetes. It is desirable that the antidiabetic agents lower the blood glucose level by inhibiting these major two enzymes delay the absorption of carbohydrates and suppress the rise of postprandial blood glucose level [7-8]. Acarbose is the hypoglycaemic agent which was used to control the diabetes by inhibiting the action of alpha amylase [9-11]. A commercially available drug such as acarbose, miglitol and voglibose inhibits the binding of oligosaccharides to alpha glucosidases [12]. It has been reported that the glycon binding subsites are the target binding sites of alpha glucosidase inhibitors. Kim et al., 1999 reported that certain inhibitors such as acarbose, acarviosine-glucose and isoacarbose were found to inhibit alpha-glucosidase, alpha amylase and CGTase (Cyclodextrin Glycosyltransferase). Among these three inhibitors acarviosine-glucose was found to inhibit 430 times more when compared to acarbose [13]. α-Glucosidase inhibiting activities of series of tetrachlorophthalimide derivatives have been reported [14]. Many plant and microbial extracts with α-glucosidase and α-amylase inhibitory activities have been reported. But the purification of the compounds from plant and microbial extracts in pure form is difficult (due to the more no of compounds presents in it) and often isolation of these compounds in pure and single isomeric form requires laborious and tedious process. Chemical synthesis of these molecules overcomes the difficulty of isolation from their natural sources.

N-chloro compounds were found to be versatile reagents and starting materials for the synthesis of various chemical and biologically important compounds [15-19]. Many of these derivatives are known to possess antimicrobial activity and reduced potential for the development of antibiotic resistance [20]. In recent years, various improved methods were reported for N-chlorination [21-22].

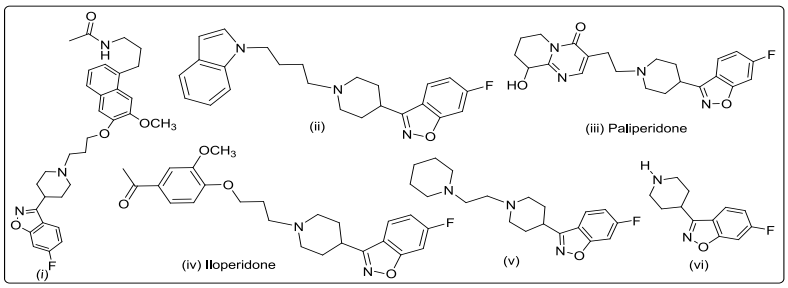
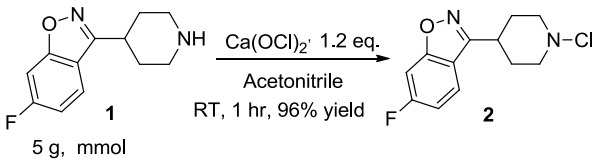


Figure 1: Some of the important benzisoxazole carrying active pharmaceutical ingredient (API's)

However the reported methods made the low chemical selectivity, involves laborious workup procedure, removal of byproducts (e.g. removal of succinamide when used as NCS reagent) and usage of the additives and monitoring the pH conditions makes the process inconvenient [23-24]. There is tremendous scope for developing new or improved to overcome this problem. Therefore it is necessary to explore the synthesis and

biological evaluation studies on N-chlorinated compounds. Recent work from our laboratory described the potential use of $Ca(OCl)_2$ as an oxidizing agent for various organic transformations such as chlorination and oxidation of alcohols [21-22 and 25].

As per the reported method on derivatives of benzisoxazole are used to treat psychological diseases (Fig.1, i) [26], inhibition of histamine receptors (Fig.1, ii) [27], used as anti psychotic drugs (Fig.1, iii and iv) [28], cholinesterase inhibitors (Fig.1, v) [29], In Fig.1, compound vi used as key intermediate for the production of drugs like iloperidone, paliperidone and resperidone [30]. In Benzisoxazole, chromans skeleton and fluorine atom substitution can alter the chemical properties and biological activities of drugs. Each substituent may have its particular merits in any given case of a biologically-active molecule [31]. In the present study we are reporting the first time, synthesis of novel N-chloro derivatives using calcium hypochlorite and its antioxidant, alpha amylase and alpha glucosidase inhibitory activities.



Scheme 1: N-chlorination of 6-fluoro-3-(piperidin-4-yl) benzisoxazole using various solvents and $Ca(OCl)_2$

RESULTS AND DISCUSSIONS

Keeping in view the importance of benzisoxazole as a model substrate, current study focuses on the possibility of N-chlorination reaction with 1.2 equivalents of calcium hypochlorite in acetonitrile under mild reaction conditions. This procedure is very simple, mild, clean, and works efficiently without any additives and produces excellent yield (96%). Our initial studies were focused on optimizing the suitable solvent system and the amount of oxidizing agent using one mmol of compound 1 with the various solvents were screened such as acetonitrile, dichloromethane, ethyl acetate, acetone, toluene, hexane, chloroform, methanol and dimethyl formamide. Among these acetonitrile was found to be the better solvent system which gave >99% conversion (confirmed by 1H NMR) and 96% of isolated yield as compared to other solvents. This is the first ever report on the synthesis of derivatized N-chloro benzisoxazole 1 and the crystal structure of compound 1 are shown in Figure 2.

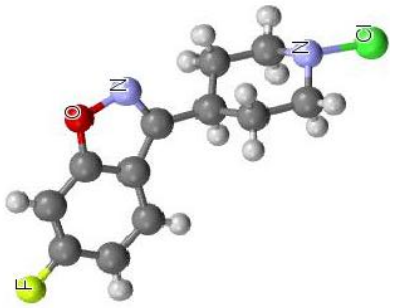


Figure 2: Single crystal ORTEP diagram of the 3-(1-chloropiperidin-4-yl)-6-fluoro benzisoxazole 2

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Biological Activities of compound 2

Anti-oxidant by DPPH Scavenging assay

DPPH radical scavenging activity of compound **2** is given in figure 2. The antioxidant activity was observed in dose dependent manner. The IC_{50} value was found to be 107 µg/mL. There were reports on heterocyclic compounds with halogen substituted (Cl^- or Br^-) patterns showing greater antioxidant activity [32]. Antioxidant activity of methoxy substituted phenyl rings exhibited antioxidant activity with IC_{50} value of 42.74 µM to 60.28 µM [33]. Inami and coworkers were recently reported that introducing the chlorine atom into the various 6-chromanol derivatives enhanced the radical scavenging activity thereby enhancing it's the antioxidant activities of the compounds [34]. In the present study we have also substituted chlorine atom in our compound, which might be the reason for significant antioxidant activity. Notably the compound **1** did not show any anti oxidant property. This is the first report on the antioxidant activity of compound **2**. Maximum inhibition (64.85%) was found at 500 µg/mL. The values were significant at $P<0.005$ with 95% confidence level.

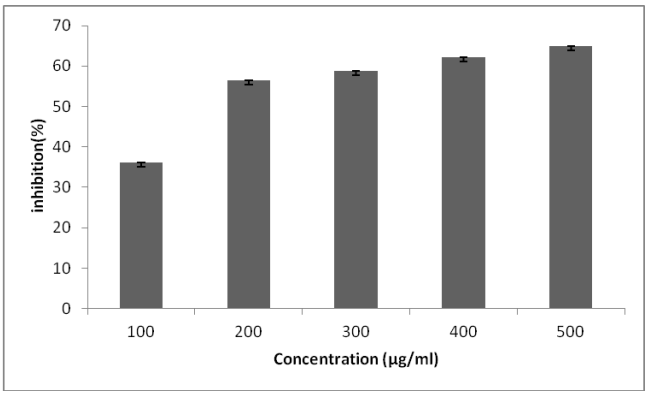

Figure 3: antioxidant activity of compound 2. The IC 50 value was found to be 107 µg/mL

Alpha amylase and alpha glucosidase inhibition

Compound 2 showed significant and notable inhibition of α-amylase and α-glucosidase. Maximum α-amylase inhibition was found to be at 500 µg/mL (61.58%). The IC50 value was found to be 270 µg/mL. The percent inhibition varied from 61.58% to 31.74% from highest (500 µg/mL) to lowest concentration (100 µg/mL). Similarly maximum α-glucosidase inhibition was found to be at 100 µg/mL (75.86%). The IC50 value was found to be 37 µg/mL. Dose dependent inhibition was noticed with both α-amylase and α-glucosidase. The percent inhibition of α-glucosidase was varied from 75.86% to 5.17% from highest (50 µg/mL) to lowest concentration (10 µg/mL). This is the first report on the α-amylase and α-glucosidase inhibitory activity of the compound 2. These enzyme inhibitors are important in the treatment of type 2 diabetes by delaying carbohydrate digestion which causes a reduction in glucose absorption rate and lowers post prandial serum glucose levels (Eichler, 1984). These drugs when taken with food, delays the absorption of carbohydrates. The main advantage of these inhibitors is that there is no alteration in the amount of carbohydrate being absorbed, so that there will not be any nutritional loss (Jong sang et al.,

2000). The values were significant at P<0.005 with 95% confidence level. The compound 2 possess significant antidiabetic activity as tested in vitro, thus the compound could be studied further for its antidiabetic property and its molecular mechanism using cellular and animal models.

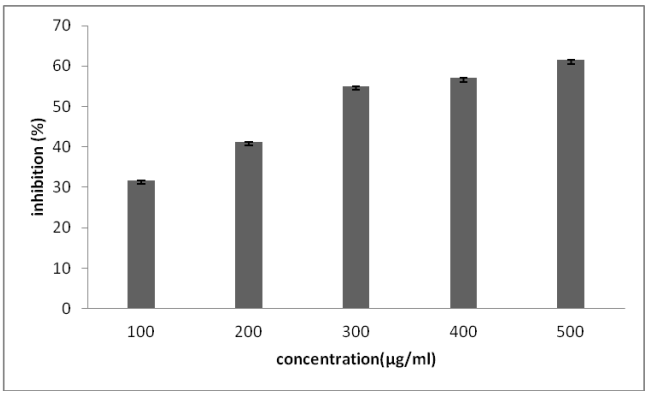

Figure 4: *alpha amylase* inhibitory activity of compound 2. The IC_{50} value was found to be 270µg/mL Values are mean ± SD

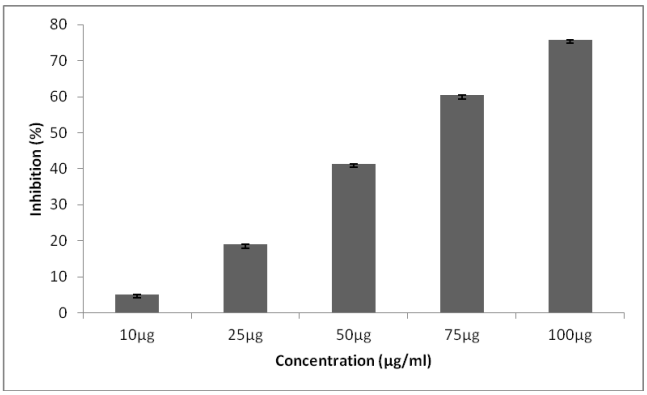

Figure 5: alpha glucosidase inhibitory activity of compound 2. The IC_{50} value was found to be 37µg/mL. Values are mean ± SD

CONCLUSION

This is the first ever report on *in vivo* of biological activity of 3-(1-chloropiperidin-4-yl)-6-fluorobenzisoxazole **2**. Current study shows the remarkable antioxidant activity, *alpha amylase* and *alpha glucosidase* inhibitory activity of compound **2**. Hence the compound **2** can be considered as potential antidiabetic drug candidates after further safe *in vivo* studies. The parent compound **1** which is key intermediate for the production of various bioactive compounds such as iloperidone, paliperidone and resperidone. Surprisingly, does not show any bio activities performed in the present study.

EXPERIMENTAL

Chemistry

The materials were purchased from Sigma-Aldrich, Merck and were used without any additional purification. All reactions were monitored by thin layer chromatography

(TLC). Melting points were recorded on an Elchem digital melting point apparatus in open capillaries and are uncorrected. The ^{1}H NMR was measured on a Bruker Avance-400 MHz instrument at room temperature. The ^{1}H NMR was measured for ~0.03 M solutions in $CDCl_3$ using TMS as internal reference. The accuracy of the ^{1}H shifts is considered to be 0.02 ppm. The coupling constants *J* are in Hertz. Mass spectra were obtained using ESI mass spectrometry.

General procedure for the N-Chlorination of 6-fluoro-3-(piperidin-4-yl) benzisoxazole (Scheme 1)

Charged 6-fluoro-3-(piperidin-4-yl) benzisoxazole (5 g, 22.7 mmol) into 50 mL of acetonitrile at 0°C. Slowly added calcium hypochlorite (3.9 g, 27.3 mmol) over ten to twenty minutes. Completion of the reaction was monitored by TLC. Filtered the salts, washed with acetonitrile, dried and concentrated under vacuum. Crude solid was purified by column chromatography to give 3-(1-chloropiperidin-4-yl)-6-fluoro benzisoxazole **2** in 96% (5.54g, 21.8mmol) as pale yellow color solid. The structure of the N-chloro benzisoxazole was confirmed from their spectral data from NMR, ES Mass and single crystal XRD.

Mp: 81-83^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz) d (ppm): 7.71-7.08 (m, 3H), 3.65 (d, 2H), 3.22 (t, 3H), 2.36-2.15(m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) d (ppm): 165.3, 163.9, 162.8, 159.9, 122.6, 122.2, 122.1, 116.9, 112.4, 97.6, 97.4, 32.9; MS (ESI) m/z Calcd: 254.1, found: 253 (M-1)**;** Crystallographic data for N-chloro benzisoxazole derivative: Mol. Formula:$C_{12}H_{12}ClFN_2O$; CCDC reference number is 878706; Intensity data were collected on an APEX CCD diffract meter equipped with Mo–Ka (l = 0.7107 A°) radiation; Cell length a =5.8979(4); Cell length b=10.4965(7); Cell length c=19.1492(12); Cell Angle α=90.0; Cell Angle β=91.783; Cell Angle γ=90.0; Cell Volume=1184.90(11); The crystallographic data for N-chloro benzisoxazole have been deposited with the Cambridge Crystallographic Data Centre. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [Fax: 44(1223)336033, E-mail: deposit@ccdc.cam.ac.uk or http:// www.ccdc.cam.ac.uk].

Biological activity Experimental Procedures

Evaluation of antioxidant activity using DPPH model:

The radical scavenging activity was determined using DPPH as described in the literature [33]. Briefly, 2 mL of 0.3 mM alcoholic solution of DPPH was added to 2 mL of compound **2** with varying concentrations (100, 200, 300, 400 and 500 µg/mL). The samples were kept in dark for 30 minutes after which the optical density was measured at 518 nm. The radical scavenging activity was determined by following formula-

$$AA\ \% = (A_{control} - A_{sample})/A_{control} * 100$$

Where, A control, is the absorbance of free radical alone and A sample is the absorbance of the free radical in the presence of compound **2**. The optical density of samples was measured against methanol which was taken as blank.

Evaluation of α-amylase inhibitory activity

The α-amylase inhibitory activity was investigated as described in the literature [35]. 500 µL of compound **2** with various concentrations (100, 200, 300, 400 and 500 µg/mL) was mixed with 500 µl of porcine α-amylase solution (0.5 mg/mL) prepared in 0.02 M sodium phosphate buffer (pH-6.9 with 0.006M sodium chloride) and incubated for 10 min at 25°C. After 10 min, 500 µL of 1% starch solution in 0.02M sodium phosphate buffer was added to each tube at 5s intervals. The reaction mixture was kept at 25°C for 10 min and finally the reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were kept in boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water and absorbance was measured at 540 nm. % inhibition was calculated by formula-

$$\% \text{ inhibition} = [A_{540\ control} - A_{540\ extract}] \times 100/A_{540\ control}$$

Evaluation for α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity was investigated as described in the literature [34]. 50 µL of compound **2** with different concentrations (10, 25, 50, 75, 100 µg/mL) were mixed with 100 µL of yeast α-glucosidase prepared in 0.1M phosphate buffer solution and kept in 96 wells plate at 25°C. After 10 min, 50 µL of 5 mM p-nitro phenyl α- D-glucopyranoside solution in 0.1 M phosphate buffer was added to each well at 5s intervals. The reaction mixture was then incubated at 25 °C for 5 min. then the absorbance was measured at 540 nm by micro-plate reader and compared with control which had 50ul of buffer solution in place of compound **2**.

The inhibitory activity was calculated by this formula

$$\%\text{inhibition} = [(A_{control540} - A_{extract540})] * 100/A_{\ control\ 540}$$

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REFERENCES

[1] Mooradian, AD, Failla, M, Hoogwerf B, Maryniuk M, Wylie-Rosett. J. Diabetes Care 1994; 17; 464-479.

[2] Mooradian AD, Micronutrients in diabetes mellitus. Ioannides C, Lewis DFV eds. Drugs, Diet and Disease Mechanistic Approaches to Diabetes. New York, NY Ellis Horwood. 1995; *2*; 183-200.

[3] Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Chiasson JL, Garg A, Holzmeister LA, Hoogwerf, B, Mayer-Davis, E, Mooradian, AD, Purnell JQ, Wheeler M. Diabetes Care 2002; 25; 148-198.

[4] Bashan N, KovsanJ, Kachko I, Ovadia H, Rudich A. Physiol. Rev 2009; 89; 27-71.
[5] Inzucchi SE. JAMA, 2002; 287; 360-372.
[6] Cheng AYY and Fantus IG. Can Med Asso J 2005; 172 ;213-22.
[7] Gin H and Rigalleau V. Diab Metabol 2000; 26; 265-272.
[8] Bhandari MR, Jong-Anurakkun N, Hong G Kawabata. J Food Chem 2008; 106; 247-252.
[9] Ali H, Houghton PJ, Soumyanath A. J Ethnopharmacol 2006; 107; 449-455.
[10] Funke I, Melzig MF, Braz J Pharmacogn 2006; 16; 1-5.
[11] Ye F, Shen Z, Xie M, Phytomedicine 2002; 9; 161-166.
[12] Kornfeld R, Kornfeld S. Ann Rev Biochem 1985; 54; 631-664
[13] Kim MJ, Lee SB, Lee HS, Lee SY, Baek JS, Kim D, Moon TW, Robyt JF, Park, KH. Arch Biochem Biophys 1999; 371; 277-283.
[14] Subramanian R, Asmawi MZ. Sadikun A. Acta Biochimica Polonica 2008; 55; 391–398.
[15] Sou S, Mayumi S, Takahashi H, Yamasaki R, Kadoya S, Sodeoka M, Hashimoto Y. Bioorg Med Chem Lett 2000; 10; 1081-1084.
[16] Priya V and Mathiyalagan N. Asian J Chem 2011; 23; 1871-1872.
[17] Alan, RK, Suman Majumder, Ritu. J Arkivoc 2003;12; 74-79.
[18] Gupta AK, Acharya J, Pardasani D, Dubey DK. Tetrahedron Lett 2007; 48; 767-770.
[19] Sathe M, Karade H, Kaushik MP. Chemistry Lett 2007; 36; 996-997.
[20] Veisi H, Ghorbani-Vaghei R, Zolfigol MA. Org Prep Proc Int 2011; 43; 489-540.
[21] Reddy CBR, Reddy SR, Shivaji Naidu, Jaya Chandra R, Muralidhar B, Sabiah S. (communicated to Arabian J chem). All synthetic methods references cited therein.
[22] Reddy SR and Chadha, A. RSC Advances 2013; 3; 14929-14933.
[23] Tilstam U, Weinmann H. Org Process Res Dev 2002; 6; 384-393.
[24] Bachand C, Driguez H, Paton JM, Touchard D, Lessard J. J Org Chem 1974; 39;3136-3138.
[25] Reddy SR, Stella S, Chadha A. Synth Commun 2012; 42; 3493-3503.
[26] Gan Y, Zhu C, Yang Q, Wang J, Zheng X, Gan Li, Zhang X. China Patent, CN 101,711,752 A, May 26, 2010.
[27] Li J, Wang G, Zhang G, Lv N, Jiao G, Liu S, Zhou S. WO 2010072147 A1, Jul 1, 2010.
[28] Strupczewski JT, Allen RC, Gardner BA, Schmid BL, Stache U, Glamkowski EJ, Jones MC, Ellis DB, Huger P, Dunn RW. J Med Chem 1985; 28; 761-769.
[29] Rangappa KS and Basappa. J Phys Org Chem 2005; 18; 773-778.
[30] Strupczewski JT, Bordeau KJ, Chiang Y, Glamkowski EJ, Conway PG, Corbett R, Hartman HB, Szewczak MR, Wilmot CA, Helsley GC. J Med Chem 1995; 38; 1119-1131.
[31] Ranganatha SR, Vinaya K., Chandrappa S, Ananda Kumar CS, Benaka Prasad SB, Prasanna DS, Rangappa KS. International Journal of Drug Design and Discovery 2010;1;-57-64.
[32] Hossain MM, Shaha SK, Aziz F. Bangladesh Med Res Counc Bull 2009; 35; 49-52.
[33] Hanif M, Shoaib K, Saleem M, Hasan Rama N, Zaib S, Iqbal J. ISRN Pharmacology 2012; 928-901.
[34] Inami K, Iizuka Y, Furukawa M, Nakanishi I, Ohkubo K, Fukuhara K, Fukuzumi S, Mochizuki M. Bioorg Med Chem. 2012; 20; 4049-4055.
[35] Jayasri MA, Radha A, Mathew TL. J Herbal Med Toxicol 2009; 3; 91-94.