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The Interaction of Piroxicam with Neutral (HP-β-CD) and Anionically Charged (SBE-β-CD) β-cyclodextrin

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Abstract

The interaction of piroxicam (PX) with sulfobutylether-β-cyclodextrin (SBE-β-CD) was studied by fluorescence spectroscopy and compared with that of hydroxypropyl-β-cyclodextrin (HP-β-CD). The stability constants (*K*) values for the PX-CDs complexes were obtained by steady-state fluorescence measurements. Inclusion conditions including concentrations of the two cyclodextrins and pH values were investigated for the complex formation in detail. The results suggested that the interaction of PX with charged CD (SBE-β-CD) is much stronger than that with uncharged CD (HP-β-CD) at any pH studied, in terms of a synergetic effect of hydrophobic and additional electrostatic interactions.

Introduction

The formation of non-covalently bound inclusion complexes between drug and cyclodextrins(CDs) is of great interest to the pharmaceutical industry, as they may improve the solubility, stability and bioavailability of guest molecule [1]. Cyclodextrins are cyclic oligosaccharides, which occur in nature as by-products of the metabolism of starch by the thermophilic bacterium, *Bacillus macerans* [2]. The most common cyclodextrins are made of six (α), seven (β) or eight (γ) glucose units bound by 1,4-ether linkage, and guest molecules can be included in their relatively hydrophobic cavities [3–10]. The complexation phenomenon often results in remarkable variations in photophysical and photochemical properties of guest molecules because of the microenvironmental difference between the CD interior and the aqueous medium [11]. This property leads to widespread applications involved in the field of improving the stability of drug against chemical and photochemical degradation [12] and controlling drug release [13] and so on, which are very interesting from a pharmaceutical point of view. Recently, a number of chemically modified CDs have been prepared to improve the inclusion capacity and the physicochemical properties of parent CDs. Of the CDs derivatives, hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutyl ether β-cyclodextrin (SBE-β-CD) have been reported on account of their high solubility in water and minimal toxicity [14]. Especially SBE-β-CD has been recently been used to stabilize drugs and is currently undergoing extensive chronic safety assessment [15].

Piroxicam(PX) [4-hydroxy-2-methyl-N-2-pyridine-2H-l,2-benzothiazine-3-carboxamide-1,1-dioxide], one of the oxicam family, is a non-steroidal anti-inflammatory drug (NSAID) with the optical properties that also possesses analgesic and antipyretic properties [1]. It is recommended for the treatment of rheumatic disorders and other chronic arthritic symptoms [16]. Besides their primary functions, oxicam group of drugs shows other diverse properties. These include chemoprevention and chemosuppression of various cancer cell lines [17] and UV induced photosensitization of the skin [18]. However, it has been associated with gastrointestinal side effects. It is possible to minimize these side effect by developing drug carriers to prevent the direct contact of drug with gastric mucosal. Thus, numerous studies focus on the inclusion complex CDs with PX including spectrofluorimetric determination of PX in β-CD system [19], the interaction between CDs and PX [20, 21], the increasing rate of dissolution and absorption of the original PX based on the complex interaction [22], determination of PX ratio in β-CD complexes [16] and so on. Few literatures involved in using the anion drug carriers such as SBE-β-CD. Especially, the conformation of PX is pH dependent, which plays an important role for the inclusion complex of the drug with CDs.

In this study, the inclusion complexation of PX with negatively charged SBE-β-CD in phosphate buffer solution was investigated by the fluorescence spectroscopy method, and its inclusion capacity was compared with that of neutral HP-β-CD. The factors affecting the inclusion process are discussed in detail, which provide

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the better understanding of unique properties of anionically charged SBE-β-CD different from neutral CDs.

Experimental

Apparatus

The fluorescence measurements were performed with a F-4500 spectrofluorometer (Hitachi, Japan). Excitation and emission bandwidths were both set at 10 nm. The pH meter (pHS-2 meter) was made in the 2nd instrument factory of Shanghai in China. All experiments were carried out at 20 ± 1 °C.

Reagents

PX was purchased from Beijing Biological Identification institute in China. HP-β-CD (average MW = 1657), degree of substitution (D.S.) = 9.0. SBE-β-CD was synthesized based on the literature method [23], D.S. = 2.5. Phosphate buffer solution was used to control the pH-value of the media. All other reagents were of analytical-reagent without further purification. Doubly distilled water was used throughout.

Procedure

A 1 ml aliquot of the stock solution (1.0×10^{-4} mol l^{-1}) of PX was transferred into a 10 ml volumetric flask, then appropriate amount of 0.01 mol l^{-1}CDs (SBE-β-CD or HP-β-CD) solution was added. The pH values were controlled by 0.5 mol l^{-1} phosphate buffer solution. The mixed solution was diluted to the final volume with distilled water and shaken thoroughly, following equilibrated for 30 min at 20 ± 1 °C. All the measurements of fluorescence were made against the blank solution treated in the same way without CDs.

Results and discussion

Influence of pH

PX is an enolic acid with the p*K*a values of the enole and the 2-pyridyl-group of are 1.86 and 6.30, respectively [24]. The presence of two ionizable groups leads to pH dependent different ionic states. Figure 1 showed PX structures in aqueous solutions with different pH values [16]. Because PX only emits fluorescence in acid solutions [25], in order to attain a high and pH independent fluorescence emission, the acidic media were selected in each case according to the p*K*a values of PX i.e. pH 0.8, 3.5 and 5.5, respectively. At pH 0.8, the pyridine moiety is protonated and only the positively charged of PX was present. At pH 3.5, the coexistence of the zwitterions with PX cation is attributed to the partial deprotonation of the enolic oxygen. With increasing the pH values, the concentration of the zwitterions increases relatively up to pH = 5.5, where zwitterions dominate and behave like neutral lipophilic [16].

Figure 2 showed the effect of pH on the fluorescence spectra of PX in the presence of SBE-β-CD. The fluorescence spectra were very depended on pH values. The corresponding excitation wavelength was 330 nm at pH 0.8. With increasing the concentration of SBE-β-CD, the emission maximum wavelength of PX red shifted from 461 nm to 467 nm. At pH 3.5or 5.5, the corresponding excitation wavelength was 310 nm, the emission maximum wavelength of PX shifted from 417 nm to 428 nm or 422 nm, respectively, with increasing of the concentration of SBE-β-CD. It was noted that the fluorescence intensity enhanced dramatically at pH 0.8, which indicated that SBE-β-CD easily complexes the protonated form of the PX. The similar phenomenon for PX with HP-β-CD was observed only the fluorescence enhancement was relatively weak.

Effect of CDs concentration

The effect of CDs concentration on the fluorescence intensity of PX was examined (shown in Figure 2). The concentration of PX was fixed at 1.0×10^{-5} mol l^{-1} and the concentration of CDs varied from 0 to 6.0×10^{-3} mol l^{-1}. The fluorescence intensity of the PX was gradually enhanced with the increase of CDs (SBE-β-CD or HP-β-CD) concentration until the stable inclusion complex was formed. SBE-β-CD resulted in a more effective inclusion interaction which was due to the participation of additional electrostatic interactions. This enhancement of the fluorescence produced through

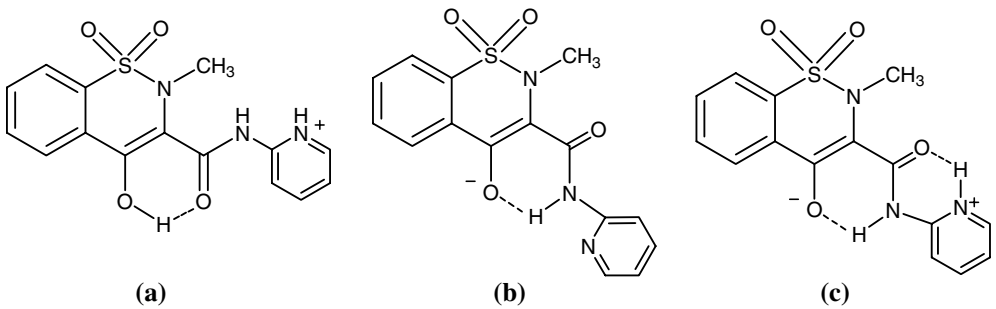

Figure 1. Piroxicam structures in aqueous solutions with different pH values (a) pH < 0.9, (b) pH > 7, (c) 0.9 < pH < 7. Structures are based on the literature [16].

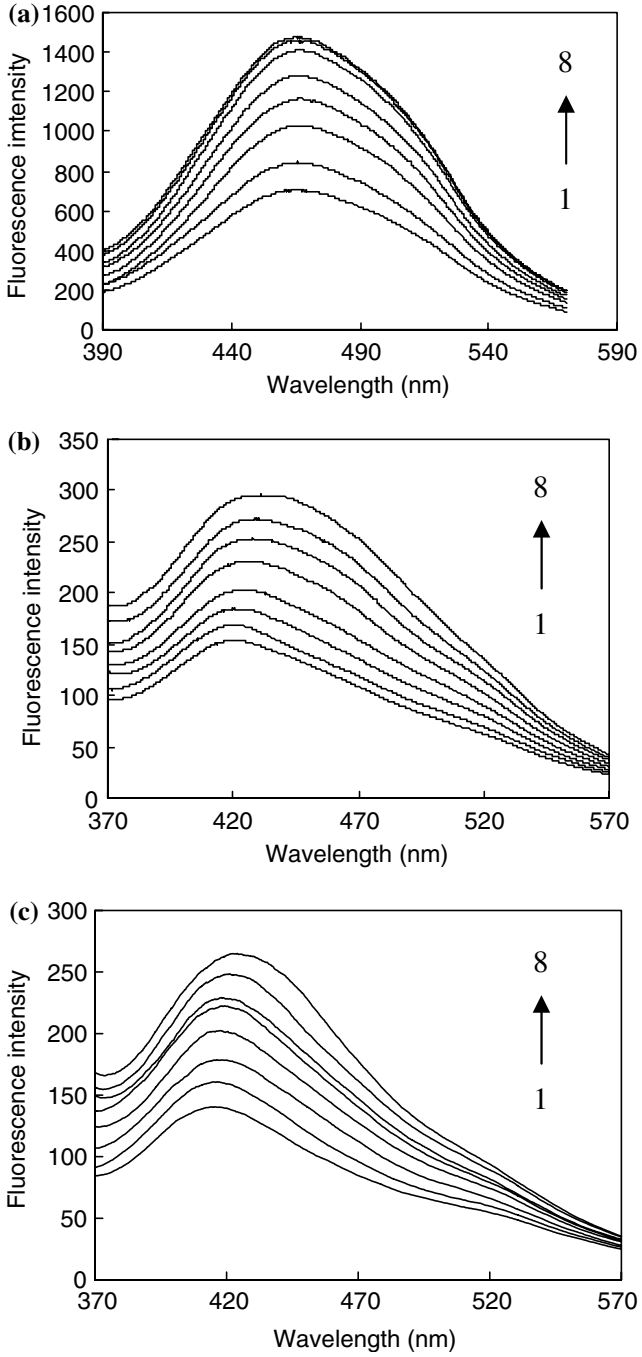

Figure 2. Fluorescence emission spectra of 1.0×10^{-5} mol l^{-1} PX at different pH values in SBE-β-CD. The pH values are (a) 0.8; (b) 3.5; (c) 5.5, respectively. SBE-β-CD concentration (mol l^{-1}): (1) 0; (2) 5×10^{-4}; (3) 1.0×10^{-3}; (4) 2.0×10^{-3}; (5) 3.0×10^{-3}; (6) 4.0×10^{-3}; (7) 5.0×10^{-3}; (8) 6.0×10^{-3}.

the formation of the complex may be very useful from an analytical point of view.

Formation constants of PX-CD complexes

Inclusion formation constant (K) power was a measure for complexes capacity of CDs. The formation constants of PX with CDs (SBE-β-CD or HP-β-CD) were evaluated at different pH values assuming a 1:1 (CD:

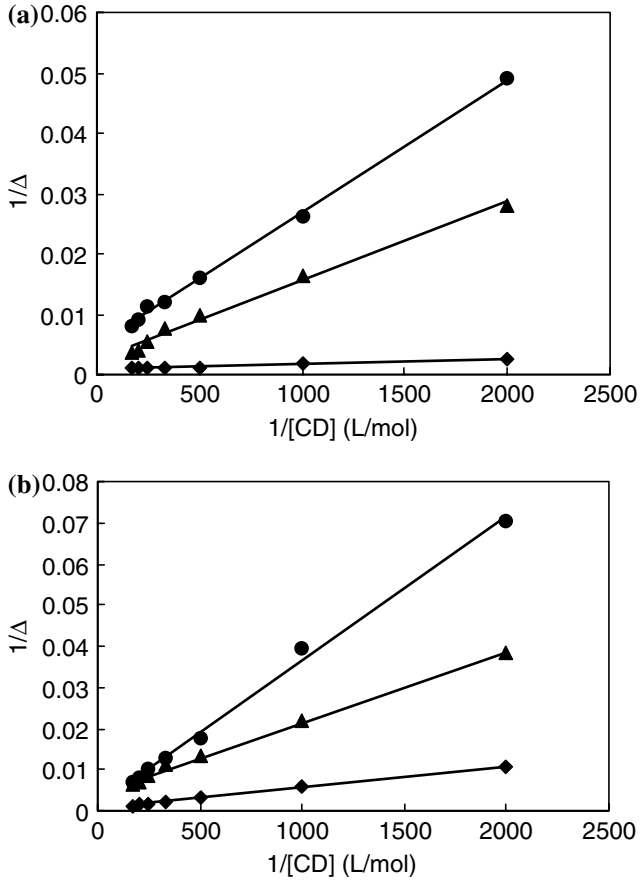

Figure 3. Double reciprocal plots for PX complexed to SBE-β-CD (a) or HP-β-CD (b) at various pH values, (♦) pH = 0.8 (●) pH = 3.5 (▲) pH = 5.5.

PX) inclusion model. The inclusion process is as follows:

$$\mathrm{CD} + \mathrm{PX} \rightleftharpoons \mathrm{CD}-\mathrm{PX} \qquad (1)$$

where the symbols CD, PX and CD-PX represent cyclodextrin (SBE-β-CD or HP-β-CD), Piroxicam, and the inclusion complex, respectively. The formation constant can be obtained by the modified Benesi-Hildebrand equation (double be reciprocal plot) [26].

$$\frac{1}{F-F_0} = \frac{1}{(KkQ[P]_0[\mathrm{CD}]_0)} + \frac{1}{kQ[P]_0} \qquad (2)$$

where F and F_0 represent the fluorescence signals of PX in the presence and absence of CD; $[P]_0$ and $[\mathrm{CD}]_0$ represent the initial concentration of PX and cyclodextrin; k is an instrumental constant; K is the formation

Table 1. Formation constants K (l mol^{-1}) for CDs-PX complexes at different pH values

Cyclodextrin	pH = 0.8	pH = 3.5	pH = 5.5
SBE-β-CD	1133 ± 155	313 ± 18	144 ± 21
HP-β-CD	99 ± 9	51 ± 15	68 ± 25

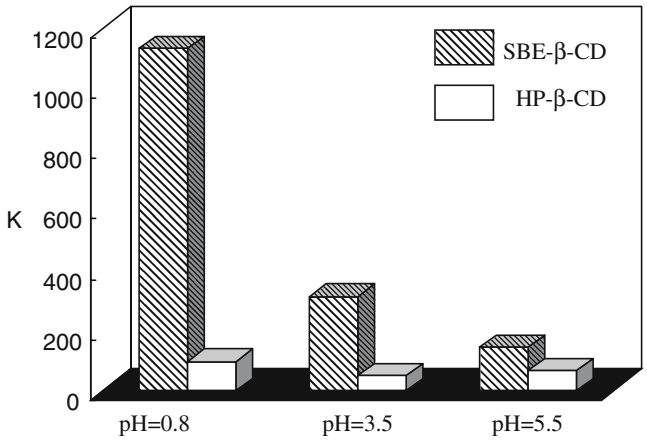

Figure 4. Formation constants for PX-CDs at different pH; pH 0.8, pH 3.5, pH 5.5.

constant of the complex; Q is the quantum yield for the complex. Figure 3a and b showed the double reciprocal plots $1/(F-F_0)$ vs. $1/[\mathrm{CD}]_0$ for PX to CDs (SBE-β-CD or HP-β-CD) in the pH range 0.8–5.5. The good linearity of the plot implied the formation of inclusion complexes with a stoichiometry of 1:1 (SBE-β-CD: PX or HP-β-CD: PX).

Formation constants of PX at different pH values in the different CD systems were listed in Table 1. The formation constant for the zwitterions forms of PX was superior to with SBE-β-CD than with HP-β-CD. For the positively charged forms, the formation constants of the PX with SBE-β-CD were significantly superior to with HP-β-CD. Figure 4 showed the PX-SBE-β-CD formation constants were more sensitive to change of pH values than PX-HP-β-CD. This implied that the selective inclusion process associated with the species form of PX and CDs.

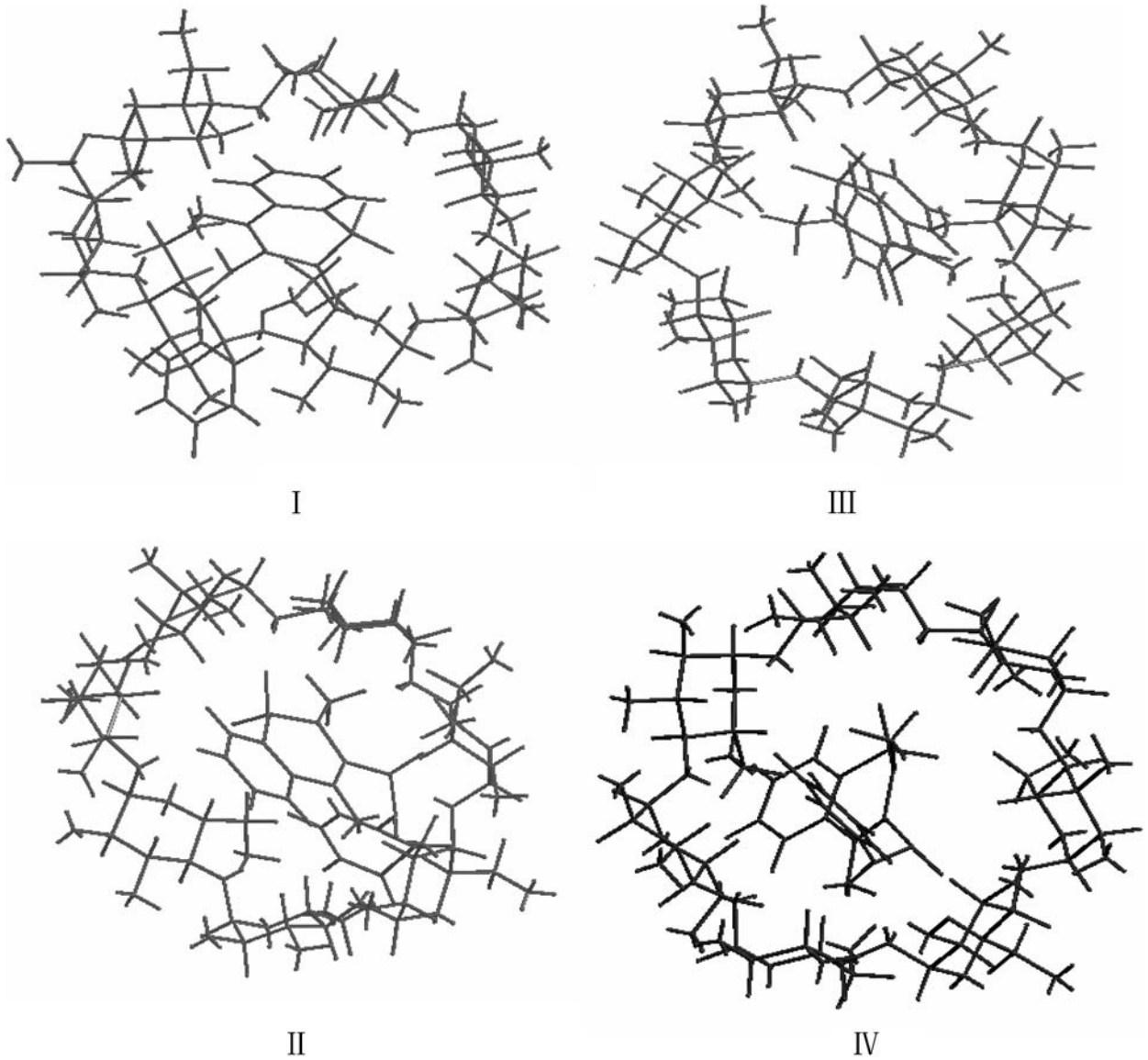

Figure 5. Molecular modeling plots of four kinds of inclusion models between CD and PX: I. The benzothiazine ring inserts into the cavity from the primary side of CD; II. The benzothiazine ring inserts into the cavity from the secondary side of CD; III. The pyridine ring inserts into the cavity from the primary side of CD; IV. The pyridine ring inserts into the cavity from the secondary side of CD.

Table 2. Energy (kJ mol^{-1}) results of conformation optimization for four kinds of CD-PX assemblies

	Mode I	Mode II	Mode III	Mode IV
Stretch	5.4033	5.3314	5.3617	5.4142
Bend	21.9115	22.1530	21.6664	20.7432
Stretch Bend	1.3738	1.2936	1.2775	1.2792
Torsion	0.8737	0.3300	0.0903	0.2024
Non−1, 4VDW	−129.1518	−109.6737	−117.1408	−122.8223
1,4VDW	61.8761	61.3673	61.5032	61.7507
Total energy	−37.7134	−19.1984	−27.2416	−33.4327

The related inclusion mechanism

One or several interactions including dipole-dipole, electrostatic, van der Waals forces, hydrogen bonding, and the release of distortion energy of CD ring upon guest binding cooperatively govern the stability of an inclusion complex [27, 28]. PX is pH dependent and exhibited different forms in acid media shown in Figure 1. HP-β-CD is not charged and hydrophobic interactions occurs between the guest and the cyclodextrin cavity and hydrogen bonding of the guest to –OH groups or other introduced groups on the CD ring. SBE-β-CD is a strong acid and carries permanent negatively charged even at a low pH [29]. It is reasonable that such effects are largely driven by a combination of electrostatic forces and hydrophobic forces with regard to the binding of positively charged PX with negatively charged SBE-β-CD. Thus, the additional electrostatic interactions of SBE-β-CD led to stronger binding properties than HP-β-CD. Moreover, inclusion complexes PX with neutral HP-β-CD is minimally affected by pH.

Molecular modeling studies

Computer molecular modeling system CS ChemDraw Ultra8.0 from CambridgeSoft Corporation being used, binding models were intimated separately from the molecular dynamic calculation. (I) The benzothiazine ring inserted into the cavity from the primary side of CD; (II) The benzothiazine ring inserted into the cavity from the secondary side of CD; (III) The pyridine ring inserted into the cavity from the primary side of CD; (IV) The pyridine ring inserted into the cavity from the secondary side of CD. From the results of the four optimized configurations (Figure 5, Table. 2), we can see that the total energy for model I was the lowest. This confirmed that the benzothiazine ring of PX penetrated into the cavity from the primary side of CD.

Conclusion

Fluorescence spectroscopy investigation has demonstrated the inclusion complexation interaction between PX and the two cyclodextrins (SBE-β-CD or HP-β-CD). Moreover, the possible spatial configuration of complex was obtained by means of molecular modeling. Among the cyclodextrins examined, anionically charged SBE-β-CD formed stronger complexes with counter-charge PX, compared to comparable complexes with neutral HP-β-CD. The additional electrostatic is different from other commonly used CDs. SBE-β-CD as drug carries showed potential application in clinic and pharmaceutical field.

Acknowledgements

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