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Studies on acridone derivatives with and without inclusion complex formation with β-cyclodextrin

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Using keto group of acridone pharmacophore, three important acridone derivatives namely thiosemicarbazone, semicarbazone and oxime have been synthesised which have significant antimicrobial activity. In order to increase the bio-accessibility of these compounds, inclusion complexes have been prepared with a non-toxic oligosaccharide, β-cyclodextrin. The synthesis of derivatives and their inclusion complexes have been ascertained from the changes in spectral characteristics and melting point data. The aqueous phase solubility studies reveal 1:1 stoichiometry between the compound and β-cyclodextrin in the inclusion complex. The calculation of thermodynamic parameters ΔG (change in free energy), ΔH (change in enthalpy) and ΔS (change in entropy) of the complexes indicates the inclusion complex formation to be spontaneous and exothermic in nature. The determination of thermodynamic stability constants suggests existence of weak intermolecular forces in between host and guest in the inclusion complex. The study of antimicrobial activity indicates that the microbes like *E. coli* and *P. aeruginosa* are susceptible to acridone and its derivatives and the susceptibility increases further after the formation of inclusion complexes.

Key words: Acridone derivatives, inclusion complex, β-cyclodextrin, phase solubility, thermodynamic stability, antimicrobial study.

INTRODUCTION:

Acridone and its derivatives are important pharmacophores for the designing of several chemotherapeutic agents (anti cancer, anti bacterial, anti protozoal) because of their strong affinity towards DNA and intercalative properties [1–3]. These compounds are suggested to be highly efficacious in preventing and treating diseases such as asthma, allergic rhinitis, atopic dermatitis, gastrointestinal allergies, *etc*. [4].

Since bio-accessibility of a drug depends upon its solubility, one of the factors limiting the pharmacological activities of acridone and its derivatives is their poor solubility in aqueous solutions [5]. The solubility of these compounds can be enhanced by forming inclusion (host-guest) complexes with β-cyclodextrin (β-CD), an easily available and less expensive encapsulator, which in turn increases drug efficiency [6].

Although a series of 10-N-susbstituted acridones, bearing alkyl side chains with tertiary amino groups at the terminal position have been reported [7], there are few reports regarding the synthesis of acridone derivatives involving the keto group.

In this paper an attempt has been made to synthesize acridone and its thiosemicarbazone, semicarbazone and oxime derivatives in their purest forms. Respective inclusion complexes of these compounds with β-CD have been synthesized. The formation of acridone, its derivatives and their inclusion complexes has been ascertained from their spectral characteristics. The stability of the inclusion complexes has been studied from thermodynamic measurements. As these compounds contain quinolone group, they are expected as potential drugs against some bacteria and accordingly antimicrobial activity studies have been made against a Gram positive bacterium, *Escherichia coli* and Gram negative bacterium, *Pseudomonas aeruginosa.*

EXPERIMENTAL

Apparatus and materials

All chemicals are procured form the local market and are of suitable Anal R grade. Double distilled water is used as the solvent for dilution. Other solvents employed are redistilled before use. The elemental analysis has been performed in a CHN analyzer. Electronic spectra are recorded on Shimadzu UV-1700 spectrophotometer while IR spectra are recorded in KBr pellets in the 400–4000 cm^{-1} region in a Shimadzu 8400 S FTIR spectrophotometer. Melting points are recorded by open

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capillary method. Antimicrobial screening by Kirby-Bauer method has been done by employing Muller Hinton agar plates in normal saline medium and sterilised cotton swabs.

Phase solubility measurements

The aqueous phase solubility of acridone and its derivatives at various concentration of β-CD has been studied by Higuchi-Connors method [8]. Accurately weighed sample of these compounds in quantities exceeding their aqueous solubility are shaken in a rotary flash shaker at room temperature with aqueous solution of β-CD in increasing concentration (0–10 mM/L) in a series of stoppered conical flask for a period of 48 hours till equilibrium is established. The solutions are filtered through Whatman No 1 paper and are analyzed in a UV-Vis spectrophotometer at 380–420 nm range. The various values of optical density (OD) at λ_{max} have been plotted against different concentration of β-CD.

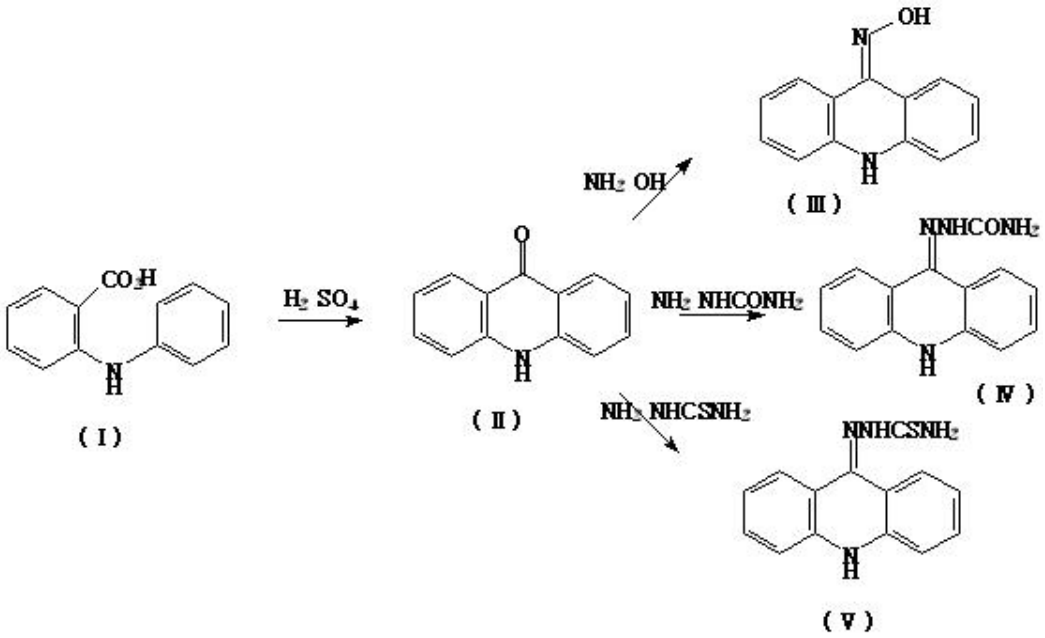
Syntheses of acridone and its derivatives

Synthesis of acridone. Acridone has been synthesised as per Allen and Mckee [9]. 0.2 mole of N-phenylanthranilic acid (I) in 100 ml of conc. H_2SO_4 is refluxed in a 500 ml flask on a boiling water bath for four hours and then poured into a 1 L flask containing hot water slowly and carefully. The yellow precipitate formed is filtered after boiling for few minutes and then the moist solid is again boiled for five minutes with a solution of 0.28 mole Na_2CO_3 in 400 ml of distilled water. The precipitate is collected with suction and washed well with water. After drying, the crude acridone (II) obtained is then recrystalised form a mixture of aniline and acetic acid.

Synthesis of derivatives. 1 g of hydroxylamine hydrochloride and 1.5 g of crystallized sodium acetate are dissolved in 10 ml water to which 0.5 g of acridone is added and shaken. Alcohol is added till turbidity disappeared to give a clear solution. Then the solution is refluxed for 2 hours on a water bath with condenser. The resulting solution is poured carefully into ice-cold water where the crystals of acridoxime (III) are obtained. These are recrystallised from alcohol and water mixture and finally dried. Similarly the semicarbazone (IV) and the thiosemicarbazone (V) derivatives have been prepared using 1 g of semicarbazide hydrochloride and 1 g of thiosemicarbazide hydrochloride, respectively. The synthesis of acridone and its derivatives are shown in Scheme 1.

Synthesis of inclusion complexes

The inclusion complexes of acridone and its derivatives have been synthesised as per co-precipitation method [10, 11]. The solution of the synthesized compounds are prepared in required concentrations (0.03M) and were added drop wise to previously stirred β-CD solution. The mixtures are stirred at room temperature for 48 hours, filtered. Then the content is and cooled for another 48 hours in refrigerator. Finally, the precipitate obtained is filtered through G-4 crucible, washed with distilled water and dried in air for 24 hours.



Scheme 1.

Study of thermodynamic properties

The thermodynamic stability constant (K_T) at room temperature of the complexes are calculated using Benesi-Hilderbrand relation [12].

$$1/\Delta A = 1/\Delta\epsilon + 1/K[\text{guest}]_0\,\Delta\epsilon.1/[\beta\text{–CD}]_0 \quad (1)$$

The stability constant K (during deencapsulation) of each complex has been calculated with increasing temperature. The slope of the linear plot of ln$K$ *versus* I/T gives rise to the calculation of ΔH (change in enthalpy) and then ΔS (change in entropy) was calculated using the integrated from of the van't Hoff equation.

$$\text{Ln}K = [-\Delta H/RT] + \Delta S/R \quad (2)$$

The value of ΔG was calculated from the value of K_T at 298 K using the equation:

$$\Delta G = -RT.\ln K_T \quad (3)$$

Study of antimicrobial activity

The disk diffusion method for antimicrobial susceptibility test is the Kirby-Bauer method [13, 14]. Muller-Hinton agar plates with normal saline medium have been used for this test. The bacterial inoculums are prepared by making a direct saline suspension of colonies of same morphological type that are selected from an 18–24 hour agar plate. The turbidity with sterile saline is adjusted. Within 15 minutes after adjusting the turbidity, a sterile non-toxic swab is dipped on an applicator into the adjusted suspension. A maximum of 5 disks on a100 mm plate are placed on the surface of the agar plate. The plates are inverted and are placed in an aerobic incubator at 35ºC. After 16–18 hours of incubation, the diameters of zones of complete inhibition (ZCI) are measured.

RESULTS AND DISCUSSION

Synthesis and characterisation of acridone and its derivatives

The synthesis of acridone and its derivatives are ascertained from elemental analysis, melting point (m.p.) measurement and changes in spectral (UV-Vis and IR) characteristics (Table 1). The elemental composition nearly matches with theoretical data. Infrared data of $C{=}O_{str}$ at 1674 cm^{-1}, $N{-}H_{str}$ at 3274 cm^{-1}, $C{-}N_{str}$ at 1161 cm^{-1} *etc*. and m.p. at 350ºC indicate the formation of acridone. Similarly, $C{=}N_{str}$ at 1560 cm^{-1}, $C{=}S_{str}$ at 1141cm^{-1}, $C{-}N_{str}$ at 1160 cm^{-1} etc and m.p. at 302ºC; $C{=}N_{str}$ at 1560 cm^{-1}, $C{=}O_{str}$ at 1635 cm^{-1}, $C{-}N_{str}$ at 1159 cm^{-1} $N{-}H_{def}$ at 1533 cm^{-1} *etc*. and m.p. at 292ºC; $C{=}N_{str}$ at 1641 cm^{-1}, $N{-}H_{str}$ at 3321 cm^{-1}, $O{-}H_{str}$ (oxime) at 3240 cm^{-1} *etc*. and m.p. at 322ºC suggest the formation of acridone thiosemicarbazone, acridone semicarbazone and acridoxime respectively.

Synthesis and characterisation of inclusion complexes

The syntheses of inclusion complexes of acridone and its derivatives are confirmed from change in melting point data, colour and spectral characteristics (Table 1). The m.p. of acridone, its thiosemicarbazone, semicarbazone and oxime derivatives are 350ºC, 302ºC, 292ºC and 322ºC respectively where as their corresponding inclusion complexes have m.p. 359ºC, 315ºC, 306ºC and 334ºC respectively. Higher m.p. values of inclusion complexes than the compounds may be due to the fact that an extra amount of thermal energy is required to bring the molecules out of β-CD cavities. Secondly, the UV-Visible absorption maxima of these compounds undergo blue shift and the peaks become broader, weaker and smoother after the formation of their inclusion complexes. Thirdly, IR frequencies due to different bonds present in the above compounds undergo a distinct downward shift towards lower energy when they form inclusion complexes. All these observations clearly demonstrate transference of the compounds from a more protic environment (aqueous media) to a less protic environment (cavity of β-CD) i.e. encapsulation of the compounds in the cavity of β-cyclodextrin. The changes in the spectral characteristics of the compounds after inclusion complex formation are attributed to development of some weak intermolecular forces like hydrogen bonding, van der Waal forces, hydrophobic interactions etc in between the host and guest molecules [15].

Phase solubility studies

The phase solubility plots of acridone and its derivatives are shown in Fig. 1. In each case, it is seen that there is a linear increase in solubility of these compounds with increasing concentration of β-CD. At a higher concentration of β-CD, a small negative deviation is observed. Since the slopes of all plots are less than unity, the stoichiometry of the inclusion complexes is 1:1 as reported by Z. Szetli [16].

The thermodynamic stability constants (K_T) of inclusion complexes are determined by the above Benesi-Hilderbrand relation (Eqn. (1)).

Good linear correlations (Fig. 2) are obtained for a plot of $1/\Delta A$ *versus* $1/[\beta\text{–CD}]_0$ for acridone and its derivatives. The values of K_T for both the complexes are calculated using the relation:

Table 1. Analytical data of acridone and its derivatives withwithout inclusion complex formation with β-cyclodextrin.

Sl No	Compound	m.p., ºC	Yield, %	Colour	Elemental analysis Found (Calculated), %				λmax nm	IR (KBr), cm^{-1}
					C	H	N	O/S		
1	Acridone	350	78	Greenish yellow	80 (80.2)	4.8 (4.6)	8.4 (8.2)	7.0 (7.18)	403, 385	1674 (C=O), 3274(N–H), 1161 (C–N), 1633 (C=C), 1572 (ring)
2	Acridone/ β-CD complex	359	81	Yellow	-	-	-	-	401, 383	1662 (C=O), 3270 (N–H), 1155 (C–N)
3	Acridonethio-semicarbazone	302	76	Bright yellow	62.66 (62.68)	4.5 (4.47)	20.81 (20.89)	11.97 (11.94)	405, 376	1560 (C=N), 1141 (C=S), 1160 (C–N), 3275 (N–H)
4	Acridonethio-semicarbazone/ β-CD complex	315	80	Yellow	-	-	-	-	403, 374	1553 (C=N), 1140 (C=S), 1156 (C–N)
5	Acridone-semicarbazone	292	78	Bright yellow	66.62 (66.66)	4.8 (4.76)	22.25 (22.22)	6.32 (6.35)	409, 381	1560 (C=N), 1635 (C=O), 1159 (C–N), 1535 (N–H)
6	Acridone-semicarbazone/ β-CD complex	306	80	Yellow	-	-	-	-	406, 379	1556 (C=N), 1633 (C=O), 1156 (C–N), 1532 (N–H)
7	Acridoxime	322	80	Bright yellow	74.31 (74.29)	4.8 (4.76)	13.3 (13.3)	7.59 (7.62)	406, 383	1641 (C=N), 3240 (O–H), 3321 (N–H)
8	Acridoxime/ β-CD complex	334	81	Yellow	-	-	-	-	403, 381	1640 (C=N), 3234 (O–H), 3315 (N–H)

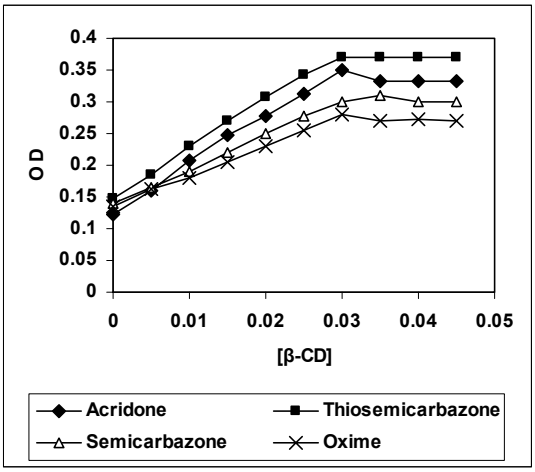


Fig. 1. Phase solubility plot (OD *vs*. [β–CD]) of acridone and its derivatives.

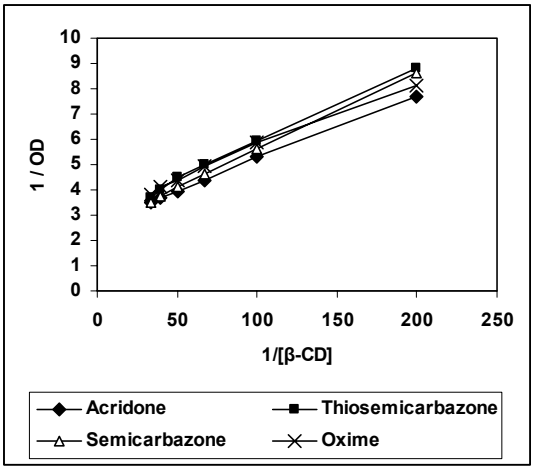


Fig. 2. Plot (1/OD *vs*. 1/[β–CD]) of acridone and its derivatives.

$$K_T = \frac{Intercept}{Slope} \quad (4)$$

The K_T values are found to be 104, 95.5, 108.3 and 155.5 M^{-1} corresponding to inclusion complexes of acridone and its thiosemicarbazone, semicarbazone and oxime. The data obtained are within 100 to 1000 M^{-1} (ideal values) indicating appreciable stabilities of the inclusion complexes [16].

Thermodynamic properties

The thermodynamic parameters associated with binding of acridone and its derivatives with β-CD for 1:1 stoichiometry have also been calculated by determining the *K* values (during deencapsulation) at different temperatures. The *K* values are found to decrease with increasing temperature (deencapsulation) as expected for an exothermic process [17]. The plot of ln*K* as a function of inverse absolute temperature produced linear plots (Fig. 3). In each case, the slope corresponds to ($-\Delta H/R$) [18]. From this value and value of K_T at 298 K, ΔG, ΔS and ΔH have been calculated (Table 2). As can be seen from the table, ΔG values are negative for all complexes. These data suggest the spontaneous formation of inclusion complexes. Secondly, the values of ΔH are negative at 298 K which suggests that the complex formation is an exothermic and enthalpy controlled process. Also, the negative enthalpy change is due to stabilization of the compound within the cavity of β-CD by weak intermolecular forces as suggested earlier. The small negative entropy change (ΔS) is

due to steric barrier caused by less free movement of guest molecules within the cavities of host. The study further suggests that change in entropy (ΔS) in destabilizing inclusion complexes is compensated by change in enthalpy (ΔH) which is in agreement with the observation of Stalin *et al*. (2006) [19].

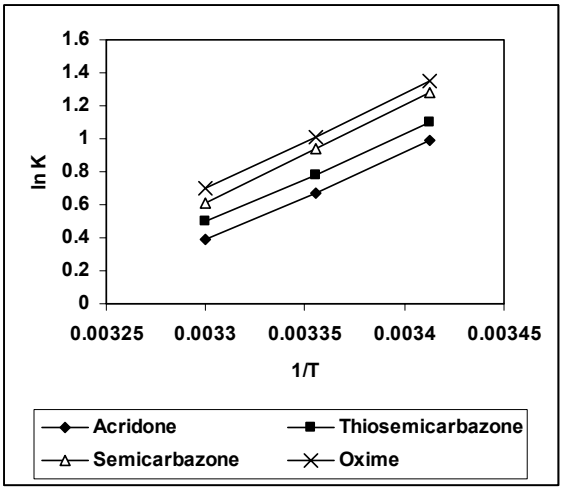


Fig. 3. Plot (1/ln$K$ *vs*. 1/T) of acridone and its derivatives.

Table 2. Thermodynamic parameters of inclusion complex of acridone and its derivatives at 298 K.

Sl No	Compound	K M^{-1}	ΔG kJ/mol	ΔH kJ/mol	ΔS kJ/mol
1	Acridone/β-CD complex	104	–11.5	–43.7	–0.11
2	Acridone-thio-semicarbazone/β-CD complex	95.5	–11.3	–31.6	–0.07
3	Acridone-semicarbazone/β-CD complex	108.3	–11.6	–46.8	–0.12
4	Acridoxime/β-CD complex	155.5	–12.5	–48.25	–0.12

Antimicrobial screening

The results obtained in the Kirby-Bauer plate method (Fig. 4) for the antimicrobial susceptibility test show that acridone and its derivatives are susceptible to both *Pseudomonas aeruginosa* and *Escherichia coli* (Figs. 5, 6).

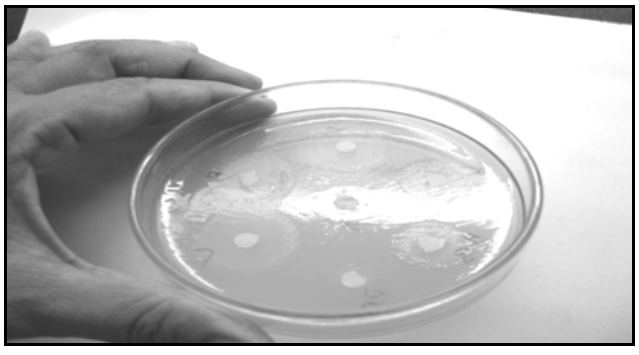


Fig. 4. Kirby-Bauer plate method of antimicrobial screening.

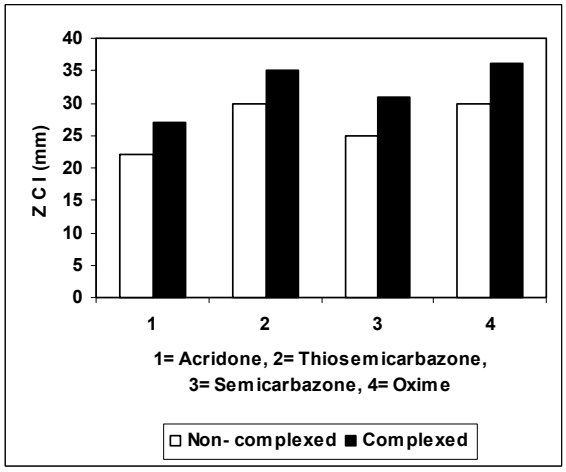


Fig. 5. Antimicrobial susceptibility test acridone and its derivatives against *P. aeruginosa*.

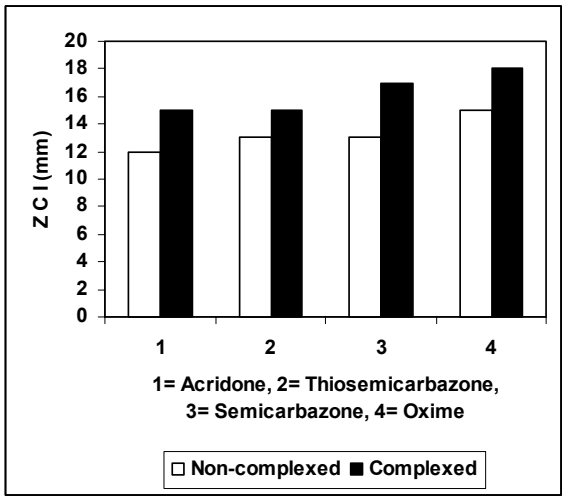


Fig. 6. Antimicrobial susceptibility test acridone and its derivatives against *E. coli*.

When inclusion complexes of these compounds are tested against the above microbes, the antimicrobial activity increases significantly. This is due to enhanced solubility of the drug which becomes more available to specific tissues leading to increased antimicrobial activity. It may be noted that the opportunistic pathogen *Pseudomonas aeruginosa* is resistant to a number of antibiotics by developing a protective biofilm around itself. But acridone and its derivatives are found to be effective against this pathogen. The drug efficiency increases further after the formation of inclusion complexes with β-cyclodextrin.

CONCLUSION

From the above results and discussions it is clear that the solubility of acridone and its derivatives can be improved by inclusion complex formation with β-CD which is a very good analytical tool for enhancing the bio-availability of drugs. The negative ΔG, ΔH, ΔS values support the formation of the

inclusion complexes. Cyclodextrins are now widely used for the stabilization of many drugs [20]. Acridone and its derivatives show antibacterial activity, which can further be enhanced by forming their inclusion complexes.

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ИЗСЛЕДВАНЕ НА ПРОИЗВОДНИ НА АКРИДОН И НА КОМПЛЕКСИ ЧРЕЗ ВКЛЮЧВАНЕ НА β-ЦИКЛОДЕКСТРИН

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(Резюме)

Синтезиране са три важни производни на акридона – тиосемикарбазон, семикарбазон и оксим – използвайки кето групата, които имат значителна антимикробна активност. За увеличаване на биологичното възприемане на тези съединения са синтезирани комплекси чрез включване на един нетоксичен олигозахарид β-циклодекстрин. Синтезът на производните и техните комплекси са потвърдени чрез промените в спектралните характеристики и данни за точка на топене. Изследванията на разтворимостта във вода показа стехиометрия 1:1 между съединението и β-циклодекстрина в комплекса. Изчисленията на термодинамичните параметри ΔG (промяна на свободната енергия) ΔH (промяна на енталпията) и ΔS (промяна на ентропията) на комплексите показва спонтанно образуване и екзотермичност на процеса. Определянето на термодинамичните стабилитетни константи предполага съществуване на слаби междумолекулни сили между гост и приемник в комплекса. Изследването на антимикробната активност показва, че микробите като *E. Coli* и *P. Aeruginosa* са чувствителни към акридон и неговите производни и чувствителността се увеличава допълнително след образуването на комплексите чрез включване.