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Synthesis and antimicrobial evaluation of urea inclusion complexes

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Abstract: In the present study a series of urea inclusion complexes **(1-30)** were synthesized and evaluated for their *in vitro* antibacterial activity against Gram positive *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus niger*. The most of the synthesized complexes have shown moderate antimicrobial activity. The urea inclusion complexes of capric acid, pamoic acid and 3-hydroxybenzoic acid were found to be the most active ones.

Keywords: Urea inclusion complexes; antibacterial activity; antifungal activity.

1. Introduction

A promising direction in the development of new effective drugs is the synthesis of molecular complexes.[1] In the presence of straight chain hydrocarbons, urea forms a hexagonal lattice with internal channels. The preparations of urea complexes of esters and alcohols have been reported by many investigators.[2-4] Htun *et al*. studied the excited proton transfer from 4-hydroxy-1-napthalenesulphonate to urea in methanol and reported the involvement of urea dimer in proton transfer reactions.[5]

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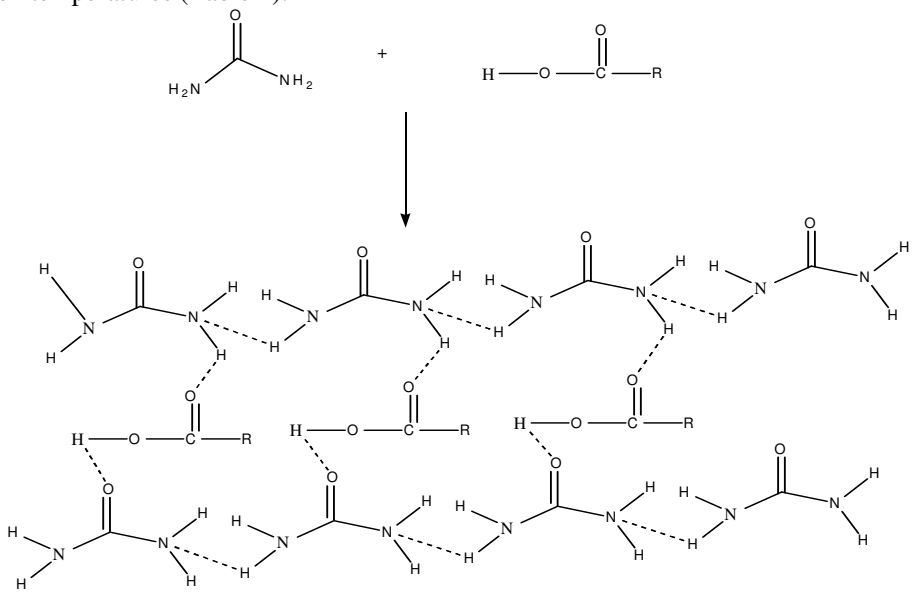
In view of above and in continuation of our previous investigations devoted to the development of antimicrobials[6-14], in the present study we have planned to characterize the antimicrobial properties of a series of urea inclusion complexes.

2. Results and Discussion

Compounds **(1-30)** were obtained by saturating the methanolic solution of organic acids with urea[15] (Scheme 1). All the synthesized complexes were characterized by their Rf values and dissociation temperatures (Table 1).



Scheme 1: Synthesis of urea inclusion complexes

The formation of urea inclusion complexes was confirmed by their IR spectroscopy (Fig. 1 – Fig. 2). The delocalization of Π electrons enhanced by donor and acceptor groups at opposite ends of the conjugated system is responsible for formation of urea inclusion complex. In the synthesized urea inclusion complexes following second order harmonic generation (SHG) active units (guests) viz. C=O, C-N of n→Π conjugation nature was present.[16] The organic acids forms urea inclusion complex due to the following facts: 1) The carboxyl group of organic acids can provide a site for hydrogen bonding; 2) The crystal structure of organic hydrogencarboxylate can incorporate a highly ordered, infinite layer of hydrogencarboxylate anions linked together by relatively short O-H….O interactions and this structure is able to organize the corresponding cation in an accentric layered or one dimensional frame work.[17]

The optimized structure of capric acid-urea inclusion complex is given in Fig. 3. This complex is optimized using AM1 method with a RMS gradient of 0.100 and a wave function of closed shell using MOPAC of Chem Office 6.0. The structure of urea inclusion complex is of tunnel shape in which the urea molecule forms a hydrogen bonded host structure that contains linear and parallel tunnels. The tunnel structure of urea is stable only in the presence of guest molecules.[18-20] It is clear from the structure of urea inclusion complex that the hydrogen bonding (N-H….N) interaction are involved in the formation of linear tunnel shaped structure of urea in which the acid molecules reside as guests due to O-H…..O interaction between carbonyl function of urea and hydroxyl function of carboxylic acid molecule.

From the data depicted in Table 2 the NH stretching of urea, which is a primary amide, appears at 3441 cm^{-1} has shifted to lower frequencies in case of stearic acid urea inclusion complex **(1)** (3409 cm^{-1}) and salicylic acid urea inclusion complex **(27)** (3415 cm^{-1}) which may be possibly due to

the hydrogen bond interaction between NH_2 of urea and OH of carboxylic acids. Similar type of results was obtained by Kremer et al.[21]

The intensity of NH stretching peak at 3409 cm^{-1} in stearic acid urea inclusion complex has decreased as compared to free urea molecules. Also the sharp NH stretching band has turned into a broad band in the stearic acid urea inclusion complex. The carbonyl group of carboxylic acid has shown variation in vibrational frequency in urea inclusion complexes in comparison to its parent molecule as well as within the urea inclusion complexes.

Table 1. Physicochemical properties of urea inclusion complexes

Compounds	R	Organic Acid used to prepare inclusion complex	Dissociation temp. (˚C)	Rf value*
1	$CH_3(CH_2)_{16}$-	Stearic acid	125-128	0.38
2	$CH_3(CH_2)_{12}$-	Myristic acid	104-107	0.35
3	$CH_3(CH_2)_{10}$-	Lauric acid	91-93	0.28
4	$CH_3(CH_2)_4$-	Capric acid	84-87	0.40
5	Ph-CH=CH-	Cinnamic acid	71-73	0.32
6	$CH_3(CH_2)_5CH(OH)CH_2CH=CH(CH_2)_7$-	Ricinoleic acid	120-123	0.02
7	(3-NO_2)Ph-	m-Nitrobenzoic acid	104-107	0.07
8	(2-COOH)Ph	Phthalic acid	82-84	0.24
9	(4-NO_2)Ph-	p-Nitrobenzoic acid	98-101	0.10
10	$H_2N(CH_2)_2CH_2$-	4-Aminobutyric acid	106-109	0.17
11	$CH_3(CH_2)_5CH_2$-	Caprylic acid	75-78	0.29
12	Ph	Benzoic acid	99-102	0.21
13	(2-Cl)Ph-	2-Chlorobenzoic acid	50-53	0.18
14	(3-OCH_3)Ph-	m-Methoxybenzoic acid	58-61	0.10
15	-	Pamoic acid	110-113	0.17
16	(4-NH_2)Ph-	4-Aminobenzoic acid	101-104	0.25
17	$CH_3CH_2CH_2$-	n-Butyric acid	70-73	0.15
18	(4-OCH_3)Ph-	4-Methoxybenzoic acid	110-113	0.09
19	(3-CH_3)Ph-	m-Toluic acid	80-83	0.13
20	CH_3CH_2	Propionic acid	62-65	0.22
21	(4-Cl)Ph-	4-Chlorobenzoic acid	118-121	0.31
22	(3-OH)Ph-	3-Hydroxybenzoic acid	97-100	0.16
23	(3,5-$NO_2)_2$Ph-	3,5-Dinitrobenzoic acid	107-110	0.19
24	(2-Br)Ph-	2-Bromobenzoic acid	90-93	0.23
25	(3,4-$OCH_3)_2$Ph-	Veratric acid	120-123	0.25
26	$CH_3CH=CHCH=CH$-	Sorbic acid	111-114	0.08
27	(2-OH)Ph-	Salicylic acid	93-96	0.11
28	HOOC-CH=CH-	Maleic acid	86-89	0.21
29	HOOC-$(CH_2)_4$-	Adipic acid	105-108	0.12
30	HOOC-CH_2-	Malonic acid	78-81	0.17

* Solvent for TLC-Toluene: Chloroform (1:3)

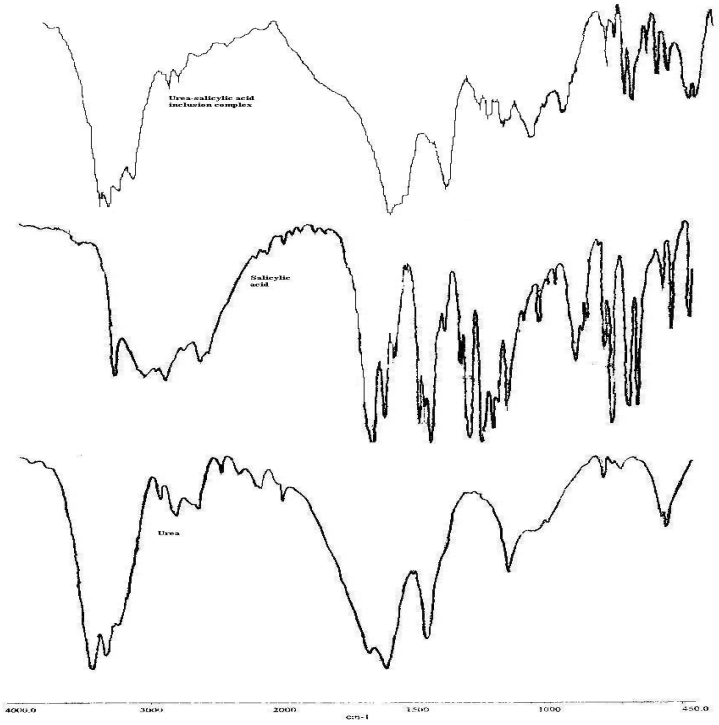


Figure 1. IR spectra of urea, salicylic acid and urea-salicylic acid inclusion complex

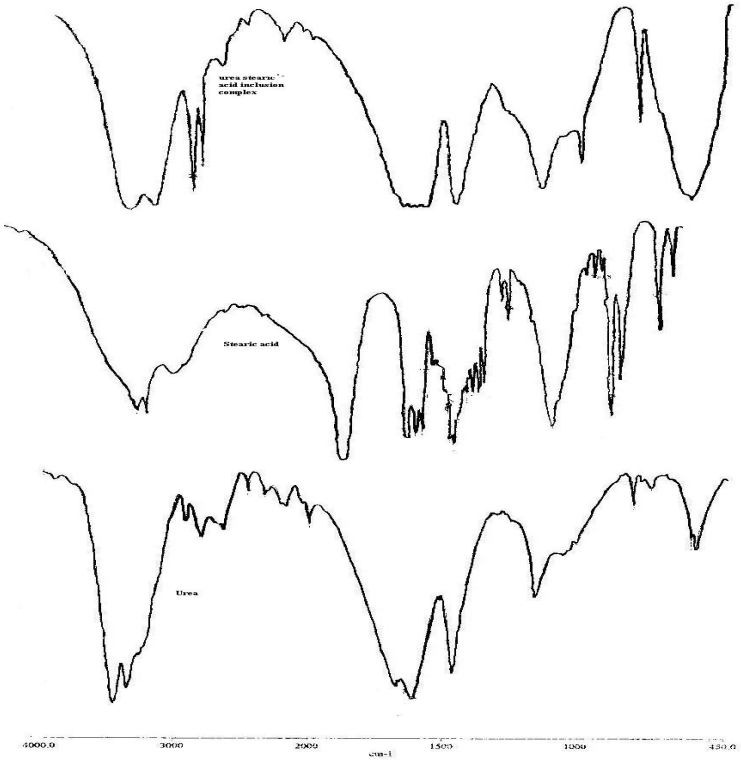


Figure 2. IR spectra of urea, stearic acid and urea-stearic acid inclusion complex

Table 2. IR data of synthesized urea inclusion complexes

S. No.	Type of vibration	Urea (cm^{-1})	SA (cm^{-1})	SALA (cm^{-1})	SAUIC (cm^{-1})	SALUIC (cm^{-1})
1	NH str., 1° amide	3441	-	-	3409	3415
2	C=O str., amide I band	1675	1699	1657	1682	1675
3	NH bend., amide II band	1617	-	-	1596	1617
4	Coupled C-N str. and NH in plane bend., amide III band	1464	-	-	1488	1465
5	O-CN deformation, Amide IV band	789	-	-	793	788
6	NH out of plane bend., amide V band	723	-	-	721	699
7	OC-N deformation, amide VI band	558	-	-	607	530
8	OH str	-	3573	3534	3655	3700
9	CH str., aromatic	-	-	3010	-	3010
10	CH str., aliphatic	-	2917 (asym.) 2850 (sym.)	-	2925 (asym.) 2952 (sym.)	-
11	Coupled CO str. and OH bend.	-	1296	1295	1295	1295
12	OH in plane bend., phenolic	-	-	1248	-	1246
13	CH bend., aromatic	-	-	1210	-	1210
14	OH bend. (COOH), out of plane	-	934	892	1013	850
15	CH bend., (1,2-disubstituted) out of plane	-	-	759	-	763

SA- Stearic acid; SALA- Salicylic acid; SAUIC- Stearic acid urea inclusion complex; SALUIC- Salicylic acid urea inclusion complex.

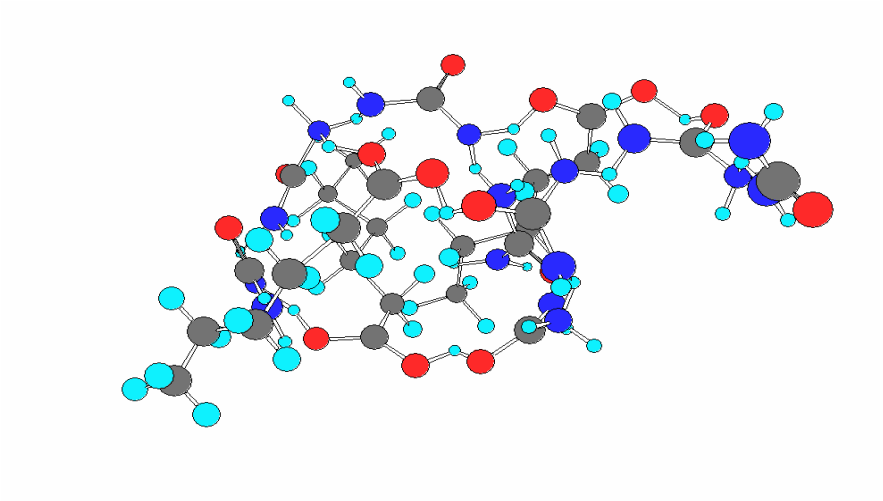


Figure 3. Optimized structure of urea-capric acid complex

In case of stearic acid urea inclusion complex, the vibrational frequency of carbonyl group has shifted to a lower frequency from 1699 cm^{-1} to 1682 cm^{-1} and in case of salicylic acid urea inclusion complex, the vibrational frequency has shifted to higher frequency from 1657 cm^{-1} to 1675 cm^{-1}. On observing these results we can assume that interactions in case of stearic acid urea inclusion complex are stronger as compared to salicylic acid urea inclusion complex which may be due to the reason that linear chain structure are better fit as guest molecule in the linear and parallel tunnels of urea.[22] In case of stearic acid urea inclusion complex, the in plane NH bending (1617 cm^{-1}) has shifted to a lower

Table 3. Antimicrobial activity of Urea inclusion complexes

Compounds	MIC (µg/mL)				
	S. aureus	***E. coli***	***B. subtilis***	***C. albicans***	***A. niger***
1	12.5	12.5	12.5	12.5	12.5
2	25.0	12.5	25.0	12.5	12.5
3	25.0	12.5	12.5	12.5	12.5
4	6.2	12.5	12.5	12.5	12.5
5	12.5	50.0	12.5	12.5	12.5
6	12.5	12.5	12.5	12.5	12.5
7	12.5	12.5	12.5	12.5	12.5
8	12.5	50.0	12.5	12.5	12.5
9	25.0	12.5	12.5	12.5	12.5
10	12.5	12.5	25.0	12.5	12.5
11	12.5	50.0	12.5	12.5	12.5
12	12.5	12.5	12.5	12.5	12.5
13	12.5	12.5	12.5	12.5	12.5
14	12.5	50.0	12.5	12.5	12.5
15	12.5	12.5	6.2	12.5	12.5
16	12.5	12.5	12.5	12.5	12.5
17	12.5	12.5	12.5	12.5	12.5
18	12.5	12.5	12.5	12.5	12.5
19	12.5	12.5	12.5	12.5	12.5
20	12.5	12.5	12.5	12.5	12.5
21	25.0	12.5	12.5	12.5	12.5
22	3.1	12.5	12.5	12.5	12.5
23	25.0	12.5	12.5	12.5	12.5
24	12.5	12.5	12.5	12.5	12.5
25	12.5	12.5	12.5	12.5	12.5
26	12.5	25.0	12.5	12.5	12.5
27	12.5	12.5	12.5	12.5	12.5
28	12.5	12.5	12.5	12.5	12.5
29	12.5	12.5	12.5	12.5	12.5
30	12.5	12.5	12.5	12.5	12.5
Ciprofloxacin	0.31	0.31	0.31		
Fluconazole				1.00	0.80

frequency (1596 cm^{-1}) whereas, in case of salicylic acid urea inclusion complex the sharp band of in plane NH bending has turned in to a broad band (1617 cm^{-1}) without any change in vibrational frequency. The NH out of plane bending (amide V band) has shifted from 723 cm^{-1} in urea to 721 cm^{-1} and 699 cm^{-1} in case of stearic acid urea inclusion complex and salicylic acid urea inclusion complex respectively.

All the synthesized complexes were tested for their *in vitro* antibacterial activity against Gram positive *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus niger*. The antimicrobial activity of synthesized urea inclusion complexes is demonstrated in Table 3. Most of the synthesized complexes have shown moderate activity against the tested strains of microorganisms.

Most of the synthesized complexes have shown a minimum inhibitory concentration (MIC) of 12.5 µg/mL. Some of synthesized complexes showed appreciable antibacterial activity as 3-hydroxybenzoic acid urea inclusion complex (**22**) was more active against the *S. aureus* with MIC of 3.1 µg/mL and the capric acid urea inclusion complex (**4**) and pamoic acid urea inclusion complex

(**15**) were more active against *S. aureus* and *B. subtilis* with MIC value of 6.2 µg/mL in comparison to other urea inclusion complexes synthesized.

3. Conclusion

In conclusion, the urea inclusion complexes were synthesized and characterized by their IR spectroscopical studies. The compounds were evaluated for their *in vitro* antimicrobial activity against the representative strains. Most of the synthesized complexes have shown moderate antimicrobial activity. The urea inclusion complexes of capric acid (**4)**, pamoic acid (**15**) and 3-hydroxybenzoic acid (**22**) were found to be the most active ones. Further modification and evaluation of the urea inclusion complexes is required to improve their potential to be chosen as an antimicrobial agent.

4. Experimental

The urea inclusion complexes were synthesized in appreciable yield and purity of synthesized urea inclusion complexes was determined by single spot TLC on silica gel G plates. All dissociation temperatures (melting points) were measured in an open capillary tube on Elico melting point apparatus and are uncorrected. The IR spectroscopy was performed on a Perkin Elmer Spectrophotometer using KBr pellets. The antimicrobial evaluation was done in duplicate.

4.1. General procedure for the synthesis of urea inclusion complexes

Organic acid (aliphatic or aromatic acid, 1g) was dissolved in 30 mL methanol. If the acid was not soluble then small amount of isopropanol was added. The above solution was saturated with urea. The solid crystals settled down were filtered off and recrystallized with methanol or isopropanol.

4.2. Antimicrobial evaluation

The antimicrobial activity was performed against Gram-positive bacteria: *Staphylococcus aureus MTCC 1430*, *Bacillus subtilis MTCC 2423*, Gram-negative bacterium: *Escherichia coli MTCC 739* and fungal strains: *Candida albicans MTCC 227* and *Aspergillus niger MTCC 2425*. The standard and test samples were dissolved in DMSO to give a concentration of 100 µg/mL. The minimum inhibitory concentration (MIC) was determined by two fold tube dilution method.[23] The dilutions of test and standard compounds were prepared in double strength nutrient broth – I.P. (bacteria) or Sabouraud dextrose broth-I.P.[24] (fungi). The samples were incubated at 37 ^{o}C (bacteria) for 24 h, 25 ^{o}C for 7 d (*A. niger*) and 37 ^{o}C for 48 h (*C. albicans*) respectively and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganism).

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