Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(5):374-381

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Study of anthelmintic properties of 1-Nicotinoyl-4-aryl-3-methyl 3a,4-dihydropyrazolo[3,4c] pyrazoles and their inclusion complexes with β-cyclodextrin

Sunakar Panda* and D. L. Singh

PG Department of Chemistry, Berhampur University, Bhanja Bihar, Odisha, India

---

ABSTRACT

Five different fused pyrazoles with nicotinoyl moiety and their inclusion complexes with β-cyclodextrin have been prepared. The formation of compounds and their inclusion complexes have been ascertained from the determination of melting point, elemental analysis and spectral properties. Finally, the compounds and their inclusion complexes have been screened for anthelmintic activities. It is found that there happens a significant increase in anthelmintic activities due to the formation of inclusion complexes.

Key words: Fused pyrazoles, β-cyclodextrin, Inclusion complexes, Anthelmintic activity

---

INTRODUCTION

Pyrazoles and fused pyrazoles exhibit a wide spectrum of biological and pharmacological activities like antifungal, antibacterial, antidepressant, antitubercular, insecticidaletc[1-9]. Secondly there are also reports that fused pyrazoles coupled with a nicotinoyl unit, are showing excellent antimicrobial activities[10]

Helminth infections are the most common health problems in India and also in other developing countries[11]. Most of the anthelmintic are used to expel parasitic worms from the body either by stunning or by killing[12] . Chemotherapeutic practices develop resistance against anthelmintic[13-15]. The drug efficiency of these compounds may be enhanced by forming inclusion complex with β- cyclodextrin, a nontoxic cheaper oligosaccharides[16,17]. Although a number of drugs are known to exhibit anthelmintic activities but there are no reports on anthelmintic activities of fused pyrazoles.

In the present work, an attempt has been made to synthesise some fused bispyrazoles with nicotinoyl unit such as of 1-Nicotinoyl-4-aryl-3-methyl 3a, 4-dihydropyrazole [3, 4c] pyrazoles in their purest forms and to prepare their inclusion complexes with β- cyclodextrin. The formation of inclusion complexes has been ascertained by the study of the physical and spectral characteristics. Finally anthelmintic activities of the compounds and their inclusion complexes are studied to know whether the inclusion complex formation has any impact on such activities.

EXPERIMENTAL SECTION

Apparatus and Materials

All the chemicals of acceptable standards were procured from local market. Double distilled water was used as solvent. Electronic spectra were recorded on Shimadzu UV-1700 spectrophotometer. IR spectra were recorded in KBr pellets in Perkin-Elmer-1800 FT-IR spectrophotometer, and ^{1}H NMR spectra (DMSO-d_6) were scanned on a DRX-300 (300MHz) spectrophotometer using TMS as an internal standard and chemical shifts are expressed in δ, ppm. Purity of the synthesized compounds has been checked by elemental analysis and homogeneity has been checked by TLC using silica gel-G, as adsorbent. Melting points were recorded by open capillary method.

Synthesis of 1-Nicotinoyl-4-aryl-3-methyl 3a, 4-dihydropyrazolo [3, 4c] pyrazoles: The synthesis of the compounds has been carried out in three steps as shown in scheme-I[10] .

i) ***Synthesis of 2-nicotinoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one:***

A mixture of nicotinic hydrazide (pyridine-3-carbohydrazide) (1.4g, 0.01mole) and ethyl acetoacetate (1.3g, 0.01mole) was taken in dry ethanol(10mL) and refluxed for 40hr. Excess of solvent was distilled off and the resultant residue was poured on crushed ice to obtain the pale white coloured residue (Compound-1).

IR (KBr): 3101(CH str., ArH), 2948 (CHstr. CH_3), 1687, 1654 (C=Ostr.), 1600cm^{-1}(C=Nstr.); ^{1}HNMR (DMSO-d_6): 7.54-8.79(m, 4H, Ar-H), 4.89 (s, 2H, CH_2), 2.26 (s, 3H, CH_3)

ii) ***Synthesis of 4-Benzylidene-2-nicotinoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one(A):***

Compound-1(0.20g, 0.001mole) was dissolved in a buffer solution of 10ml acetic acid and anhydrous sodium acetate (0.082g, 0.001mole) and benzaldehyde (0.106g, 0.001mole) was added to it. The resultant reaction mixture was refluxed for 12hr, cooled, filtered and poured on crushed ice and kept for sometimes. Solid 4-(bezylidene)-2-nicotinoyl-5-methyl-2,4-di-hydro-3H-pyrazol-3-one has been gradually appeared. It was filtered and dried **(A)**.

IR(KBr): 3101(C-Hstr., Ar-H), 2922(C-Hstr., CH), 1709(C=Ostr.), 1592cm^{-1}(C=Nstr.); ^{1}HNMR(DMSO-d_6): 7.09-8.01(m, 9H, Ar-H), 6.22(s, 1H, =CH-Ar), 2.10(s, 3H, CH_3).

Similarly, compound **B:** 4-(4-bromobenzylidene)-2-nicotino-yl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one, compound **C:** 4-(3-nitrobenzylidene)-2-nicotino-yl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one, compound **D:** 4-(2-chlorobenzylidene)-2-nicotino-yl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one and compound **E:** 4-(4-chlorobenzylidene)-2-nicotino-yl-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one have been prepared. The characteristic spectral data of the above compounds were given below:

Characteristics of B:

IR(KBr): 3093(C-Hstr., Ar-H), 2913(C-Hstr., CH_3), 1711(C=Ostr.), 1600(C=Nstr.), 738cm^{-1}(C-Brstr.); ^{1}HNMR (DMSO-d_6): 6.94-7.94(m, 8H, Ar-H), 6.26(s,1H,=CH-Ar), 2.12(s, 3H, CH_3).

Characteristics of C:

IR(KBr): 3091(C-Hstr., Ar-H), 2916(C-Hstr., CH), 1717(C=Ostr.), 1589cm^{-1}(C=Nstr.); ^{1}HNMR(DMSO-d_6): 7.07-7.99(m, 8H, Ar-H), 6.19(s, 1H, =CH-Ar), 2.17(s, 3H, CH_3).

Characteristics of D:

IR(KBr): 3093(C-Hstr., Ar-H), 2913(C-Hstr., CH_3), 1711(C=Ostr.), 1600(C=Nstr.), 738cm^{-1}(C-Clstr.); ^{1}HNMR (DMSO-d_6): 6.94-7.94(m, 8H, Ar-H), 6.26(s, 1H, =CH-Ar), 2.12(s, 3H, CH_3).

Characteristics of E:

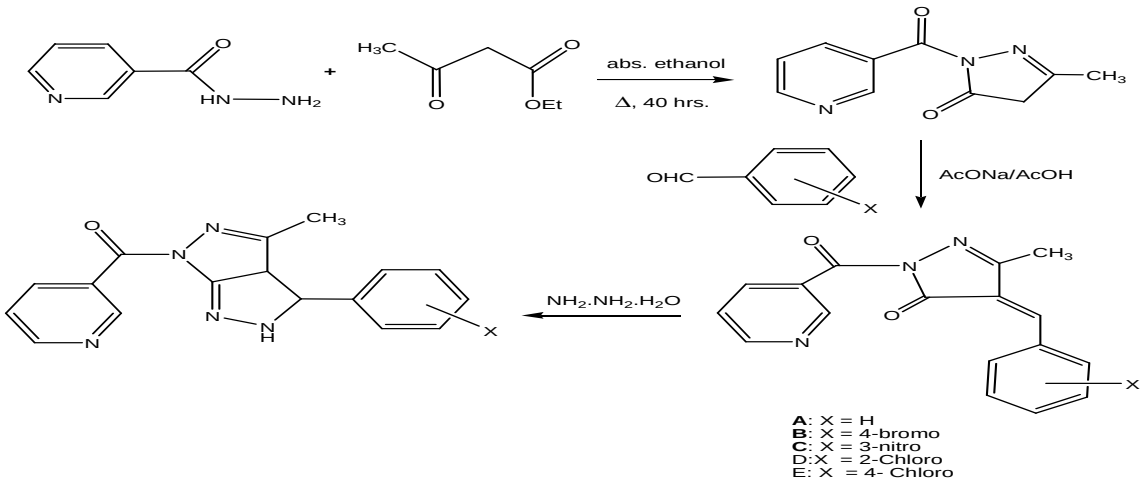
IR(KBr): 3093(C-Hstr., Ar-H), 2913(C-Hstr., CH_3), 1711(C=Ostr.), 1600(C=Nstr.), 738cm^{-1} (C-Clstr.); ^{1}HNMR (DMSO-d_6): 6.94-7.94(m, 8H, Ar-H), 6.26(s, 1H, =CH-Ar), 2.12(s, 3H, CH_3).

iii) ***Synthesis of 1-nicotinoyl-4-phenyl-3-methyl-3a,4-dihydropyrazolo[3,4-c] pyrazole*(K):**
Compound A (0.34g, 0.001mole) and hydrazine hydrate (0.002mole) were taken in dry ethanol (10mL) and a few drops of acetic acid (as catalyst) was added to it. Then the reaction mixture was refluxed for 9hr, concentrated, cooled and poured on crushed ice. The product obtained was washed several times with water and then dried **(K)**.

Similarly compound **L:** 1-Nicotinoyl-4-(4-bromobenzylidene)-3-methyl-3*a*,4-dihydropyrazolo[3,4-*c*]pyrazole, compound **M:** 1-Nicotinoyl-4-(3-nitrobenzylidene)-3-methyl-3*a*,4-dihydropyrazolo[3,4-*c*]pyrazole, compound **N:** 1-Nicotinoyl-4-(2-Chlorobenzylidene)-3-methyl-3*a*,4-dihydropyrazolo

[3,4-*c*]pyrazole and compound **O:** 1-Nicotinoyl-4-(4-chlorobenzylidene)-3-methyl-3*a*,4-dihydropyrazolo[3,4-*c*]pyrazole were prepared. Their characteristic spectral and analytical data were given in Table**1** and **2**.

SCHEME



Aqueous Phase Solubility Measurements
The aqueous phase solubility of the compounds has been studied by Higuchi-Corner method at various concentrations of β-cyclodextrin (0-10mM)[19]. Accurately weighed sample of these compounds were shaken in rotary flash shaker at room temperature by using in a series of conical flask for a period of 48 hours till the attainment of equilibrium. The solutions were filtered through whatmann-42 filter paper and were analyzed in a UV-visible spectrophotometer. The various values of absorbance at λ-max were plotted against different concentrations of β- cyclodextrin.

Synthesis of inclusion complexes
The inclusion complexes of the compounds with β-cyclodextrin have been prepared as per co-precipitation method [14-17]. Proper concentrations of the solutions of these compounds were added drop by drop to β-cyclodextrin solution of the required concentration. Stirring of the solutions was carried out for a period of 48 hours. The stirred solutions are filtered. The filtrates were cooled for 24 hours in refrigerator. The precipitates obtained were filtered, washed with water and dried in open atmosphere for 24 hours.

Evaluation of Anthelmintic activity
The synthesized compounds are screened for anthelmintic activity by using earth worms (*Peritima posthuma)*.The organism, *Peritima posthuma*, was selected because it has anatomical and physiological resemblance with intestinal roundworm parasite of human beings. Six earth worms of nearly equal size and equal weight were placed in standard drug solution and test compound solutions at room temperature. Normal saline was used as control. The standard drug and test compounds were dissolved in minimum quantity of DMSO and the volume was adjusted up to 15 mL with normal saline solution to get the concentration of 0.5% w/v. Albendazole (20mg/ml) was used as a standard drug. Poured 10 ml of each suspension in separate petri dish. Left six animals in each petridish. Observations were made for the time taken to paralyze or death of individual worms. Paralysis was said to occur

when the worms do not receive any sense even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body colour, when dipped in warm water (50^{o}C). The compounds were evaluated for the time taken for complete paralysis and death of earthworms. The mean lethal time for each test compound was recorded and compared with standard drug.

RESULTS AND DISCUSSION

The synthesis of compounds was confirmed from physical data (Table 1) and spectral data (Table 2). The elemental compositions were matching with theoretical data (Table-1). The Infra-Red and NMR data indicated the presence of expected bonds and groups in the newly synthesized compounds. The inclusion complex formation was confirmed from significant changes in colour, melting point (Table-1), a shift in UV-Visible absorption maximum and Infra-red signals of characteristic absorption peaks (Table-2). The higher melting point of inclusion complexes than the compounds may be attributed to the fact that extra amount of thermal energy is required for the latter to bring it out of β- cyclodextrin cavity. The shift in UV-Visible absorption maximum and Infra-red signals of characteristic absorption peaks (Table-2) may be attributed to the transference of the compound from a more protic environment to a less protic environment within the cavity of β-cyclodextrin. Such changes in spectral characteristics due to inclusion complex formation may be due to the weak interactions like hydrogen bonding, vander Waal's forces, hydrophobic interactions etc. between the guest compound and the host as proposed earlier [16-18,20,21]. The aqueous phase solubility plots of the compounds with in β-cyclodextrin solution (Fig. 1) exhibited a linear increase in solubility of these compounds with increasing concentration of β-cyclodextrin. Since the slopes of all the plots were less than unity, the stoichiometry of these complexes may be 1:1{21-23]. The thermodynamic stability constants (K_T) of inclusion complexes were determined by using Benesi-Hilderband relation [24]. Good linear correlations were obtained for a plot of 1/ΔA verses [β-CD] for compounds (Fig. 2). The values of K_T for all the complexes were calculated by using the following relation

K_T = Intercept/Slope

The K_T values of the inclusion complexes of compounds with β- Cyclodextrin were found to be 150, 375, 174,414 and 236 M^{-1} respectively (Table 3). The data obtained were within 100 to 1000 M^{-1}(ideal values) indicating appreciable stabilities for the inclusion complexes through host-guest interaction like van der Waal' force, hydrophobic interaction etc [25-28].

Table-1 : Physical properties of the compounds with and without inclusion complex

S. No.	Compound/ complex	Colour	Melting Point (^{o}C)	Elemental Analysis (%) (Theoretical) (Experimental)			
				C	H	N	O
1	Compound- K	Bright white	221	67.1 (67.2)	4.6 (4.7)	23.1 (23.0)	5.2 (5.1)
2	Compound- K With β-CD	Dull White	273				
3	Compound- L	Pale yellow	206	52.9 (53.0)	3.6 (3.7)	18.2 (18.2)	4.2 (4.1)
4	Compound- L With β-CD	Pale White	281				
5	Compound- M	yellow	220	58.2 (58.3)	4.1 (4.0)	24.0 (23.9)	13.7 (13.8)
6	Compound- M With β-CD	Pale yellow	288				
7	Compound- N	Bright yellow	215	60.1 (60.1)	4.1 (4.0)	20.6 (20.7)	4.7 (4.8)
8	Compound- N With β-CD	White	270				
9	Compound- O	Light brown	218	60.1 (60.1)	4.1 (4.0)	20.6 (20.7)	4.7 (4.8)
10	Compound- O With β-CD	Pale White	279				

Compound-K: 1-Nicotinoyl-4-phenyl-3-methyl-3a,4-dihydropyrazolo[3,4-c]pyrazole
Compound-L:1-Nicotinoyl-4-(4-bromobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3,4-c]pyrazole
Compound-M: 1-Nicotinoyl-4-(3-nitrobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3,4-c]pyrazole
Compound-N: 1-Nicotinoyl-4-(2-Chlorobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3,4-c]pyrazole
Compound-O: 1-Nicotinoyl-4-(4-chlorobenzylidene)-3-methyl-3a,4-dihydropyrazolo[3,4-c]pyrazole

Table-2: Spectral data of the compounds with and without inclusion complex

S. No.	Compound/ Complex	UV γ (nm)	IR (KBr) cm^{-1}	^{1}HNMR
1	Compound- K	262	3392(N-H str), 3019(C-H str, Ar),1650(C=O str), 1541(C=N str)	δ 8.78(s, 1H, NH), 7.04-7.86(m, 9H, Ar-H), 4.88-4.89(dd, 2H, CH-CH), 2.14(s, 3H, CH_3), 1.612(s), 1.427(s), 0.880(t)
2	Compound- K With β-CD	264	3401(N-H str), 1651(C=O str), 1403(C=N str),	δ 7.264(s, 1H, NH), 2.3(d), 2.329(s), 1.576(s), 0.859(t)
3	Compound- L	261	3385(N-H str), 3020(C-H str, Ar), 1650(C=O str), 1534(C=N str), 762(C-Br str)	δ 8.601(s, 1H, NH), 7.729-7.263(m, 8H, Ar-H), 2.352(s, 3H, CH_3), 1.612(s), 1.427(s), 0.880(t)
4	Compound- L With β-CD	263	3396(N-H str), 3021(C-H str, AR),1648(C=O str), 1534(C=N str), 761(C-Br str	δ 7.263(s, 1H, NH), 7.729-7.263(m, 8H, Ar-H), 2.355(s, 3H, CH_3), 2.329(s), 1.571-0.832(s), 0.880(t)
5	Compound- M	261	3411(N-H str), 3021(C-H str, Ar), 1646(C=O str), 1530(C=N str)	δ 8.85(s, 1H, NH), 7.09-7.87(m, 8H, Ar-H), 4.80-4.81(dd, 2H, CH-CH), 2.05(s, 3H, CH_3)
6	Compound- M With β-CD	262	3434(N-H str), 3019(C-H str, Ar), 1650(C=O str), 1529(C=N str)	δ 7.264(s, 1H, NH), 7.09-7.87(m, 8H, Ar-H), 4.181-4.159(dd, 2H, CH-CH), 1.574(s, 3H, CH_3)
7	Compound- N	261	3435(N-H str), 3019(C-H str, Ar), 1643(C=O str), 1565(C=N str), 771(C-Cl str)	δ 9.1(s, 1H, NH), 6.90-8.20(m, 8H, Ar-H), 4.84-4.85(dd, 2H, CH-CH), 2.16(s, 3H, CH_3)
8	Compound- N With β-CD	262	3401(N-H str), 3019(C-H str, Ar), 1650(C=O str), 1525(C=N str), 758(C-Cl str)	δ 9.1(s, 1H, NH), 6.90-8.20(m, 8H, Ar-H), 4.84-4.85(dd, 2H, CH-CH), 2.16(s, 3H, CH_3)
9	Compound- O	261	3408(N-H str), 3020(C-H str, AR), 1656(C=O str), 1565(C=N str), 758(C-Cl str)	δ 8.71(s, 1H, NH), 6.94-7.92(m, 8H, Ar-H), 4.84-4.85(dd, 2H, CH-CH), 2.16(s, 3H, CH_3)
10	Compound- O With β-CD	262	3400(N-H str), 3019(C-H str, Ar), 1650(C=O str), 1525(C=N str), 757(C-Clstr)	δ 7.26(s, 1H, NH), 6.94-7.92(m, 8H, Ar-H), 4.84-4.85(dd, 2H, CH-CH), 1.57(s, 3H, CH_3)

Table 3: Thermodynamic stability constant of inclusion complexes(K) at 298 K

S. No.	Compound/complex	K M^{-1}
1	Compound- K With β-CD	150
2	Compound- L With β-CD	375
3	Compound- M With β-CD	174
4	Compound- N With β-CD	414
5	Compound- O With β-CD	236

The of anthelmintic activities of the compounds and their inclusion complexes are studied using earth worms (*Peritima posthuma)*and the efficiency of the compounds in causing paralysis and death of earth worms are shown in Fig.3 and 4 respectively. It is seen that both the compounds and their inclusion complexes are capable of causing the paralysis and death of earth worms. However, the inclusion complexes are more efficient in causing the paralysis and death of earth worms as compared to their corresponding compounds. This may be attributed to enhanced solubility of the compounds after their inclusion complex formation which becomes more available to specific tissues leading to increased anthelmintic activity

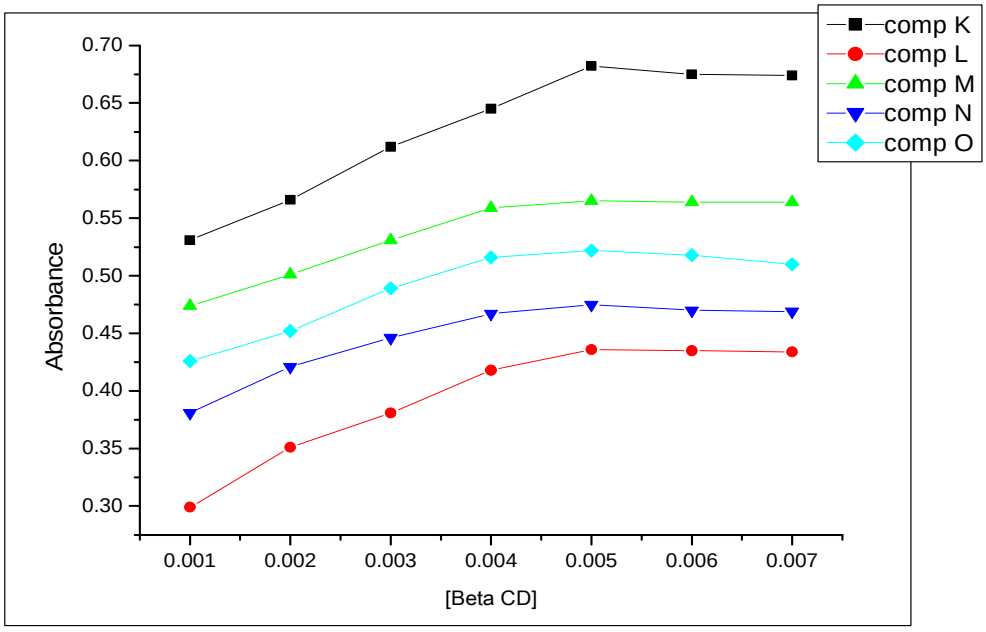


Fig. 1: Aqueous Phase Solubility of the compounds

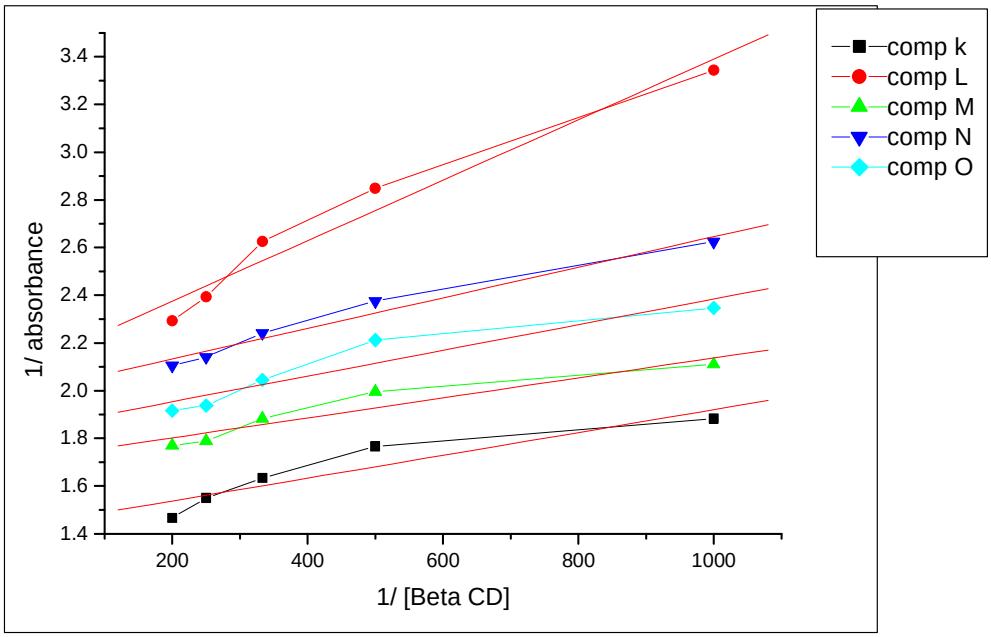


Fig. 2: Plot of 1/Absorbance Vs. 1/ [β-CD]

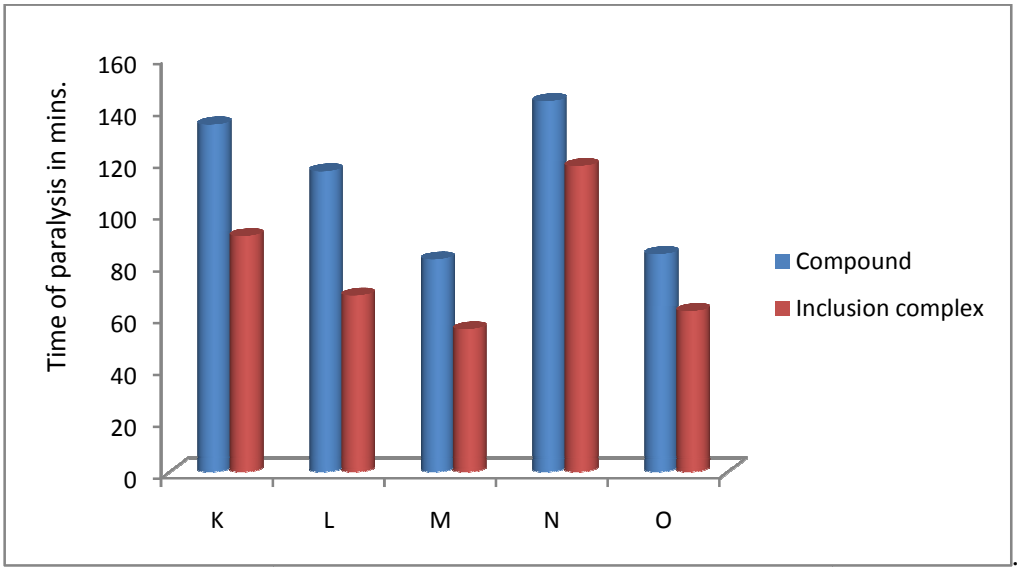


Fig. 3: Paralysis study of earthworms

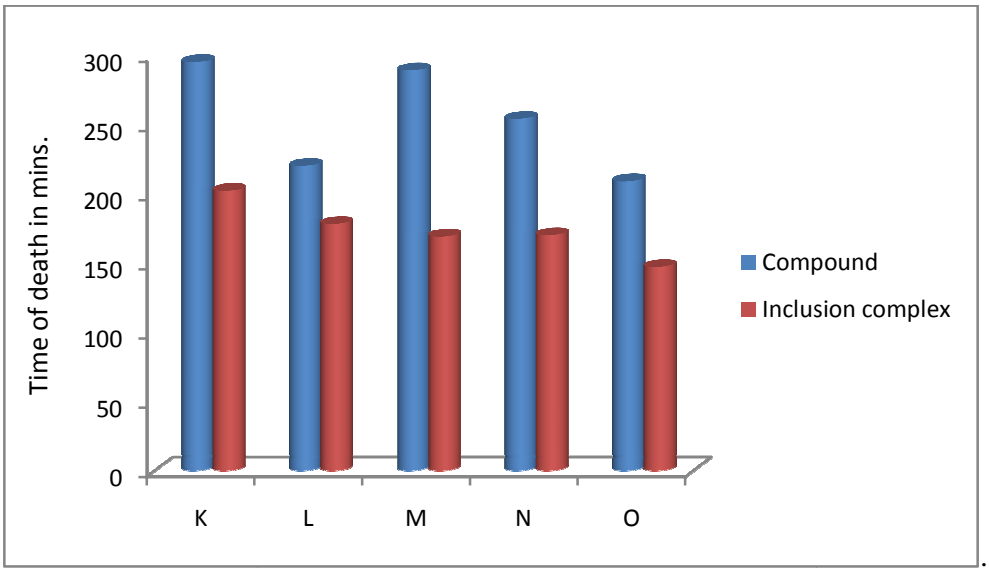


Fig. 4: Death study of earthworms

CONCLUSION

From the above results and discussion, it was clear that the formation of inclusion complexes of compounds (K, L, M, N and O) was thermodynamically allowed which can be a very good analytical tool for enhancing the bioaccessibility of the drugs. The study further reveals that the formation of inclusion complex causes a significant increase in anthelmintic activity .

Acknowledgement
The authors are thankful to Dr. J R Panda, Department of Pharmaceutical Science, Roland Institute of Pharmaceutical Sciences, Berhampur, for studying and anthelmethetic activity. Thanks to CDRI, Lucknow for analysis of sample for IR and NMR. Financial assistance from UGC is also thankfully acknowledged.

REFERENCES

[1] F. E. Goda, A. R. Maarouf and E. R. El-Bendary, *Saudi Pharm J*, **2003,**11, 111
[2] T. I. EL-Emary, *J. Chin.Chem. Society*, **2006**, 53, 391
[3] A. K. Mansoor, M. M. Eid and N. S. A. M. Khalil, *Molecules*, **2003**, 8,744
[4] S. S. Korgaokar, P. H. Patil, M. J. Shah and H. H. Parekh, *Indian J Pharm Sci*, **1996**, 58, 222
[5] E. Palaska, M. Aytemir, I. T. Uzbay and D. Erol, *Eur J Med Chem*, **2001**, 36, 539
[6] P. Y. Rajendra, R. A. Lakshmana, L. Prasoona, K. Murali and K. P. Rav, *Bioorg Med Chem Lett*, **2005**,15, 5030
[7] Z. K. H. B. Ozdemir, B. Gumusel, U. Calis and A. A. Bilgin, *Eur J Med Chem*, **2007**, 42, 373
[8] O. Ruhogluo, Z. Ozdemir, U. Calis, B. Gumusel and A. A. Bilgin, *Arzneim Forsch*, **2005**, 55, 431
[9] R. H. Udupi, A. S. Kushnoor and A. R. Bhat, *Indian J Het Chem*, **1998**, 8, 63
[10] J. A. Joshi, D. K. Sain, B. Thadhaney, S. Ojha, N. Hussain and G. L. Talesara, *Indian J Chem*, **2010**, 49B, 965
[11] P. Dabadi, B.C. Koti, A. H. M. Vishwanathswamy and Chandrakala, *Ind. J. Novel Drug Delivery*, **2011**, 3(4), 307
[12] The Merck Index, 12th Ed., page 1119: entry 6611 Nicotine, Merck & Co. **1996**
[13] R. Blakemore, Transactions of the Royal Zoological Society of New SouthWales, **1999**
[14] M.Chaturvedi, S. Dwivedi, A. Dwivedi ,P.K.Barpete and R. Sachan, *Ethnobot.Leaflet*,**2009**,13,329
[15] C.Chartier, F.Soubirse, I.Pors, A.Silvestre, J.Hubert, C.Couquet and J.Cabaret, *J.Helmithology*,**2001,**75,325.
[16] S. Panda and J. K. Tripathy. *J Chem Pharm Res*, **2010**, 2, 722
[17] S. Panda and J. K. Tripathy, *Res. J.Pharma. Tech.*, **2011**, 4, 1693
[18] S. Panda and J. K. Tripathy, *Asian J. Chem*, **2011**, 23, 1631
[19] T. Higuchi and K. Connors, *Adv. Anal. Chem. Instument*, **1965**, 4, 117
[20] S. Panda and S. S. Nayak, *Asian J. Res. Chem*, **2009**, 2(4), 539
[21] S. Panda, R. Sahu and S. K. Nayak, *J. Chem. Pharm. Res*, **2012**, 4(5), 2540
[22] S. S. Nayak, S. Panda, P. Panda and M. S. Padhy, *Bul. Chem. Comm*, **2010**, 42(2), 147
[23] S. Panda, J. K. Tripathy and J. R. Panda, *Int. J. Pharma. Sc. Drug Res*, **2012**, 4(3), 191
[24] H. A. Benesi, J. H. Hilderband, *J. Am. Chem. Soc*, **1999**, 71, 2703
[25] K. G. Mohammed and C. A. Moji, *Pharma. Dev. Tech.*, **2001**, 6, 315
[26] V. R. Bollela, D. N. Sato, B. A. L. Fonesca, *Braz J Med Biol Res*, **1999**, 32, 1073
[27] A. P. Mukna and M. S. Nagarsenkar. *American Asso. Pharma. Sc.Tech.*, **2001**, 5(1), 19
[28] J. Szetli, Molecular entrapment and release properties of drugs by cyclodextrins.Controlled Drug Bio-availability. Vol. 3, Willey Interscience publications, New York, **1985**, pp: 365