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Antibacterial activity of phenoxazine derivatives

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ABSTRACT

Phenoxazines and its derivatives have shown diverse biological activities, but investigations on antibacterial activity are limited. In the present study antibacterial activities of the synthesized hexyl series compounds of phenoxazine 10-[6'-[N-Diethyl amino]hexyl]-2-chlorophenoxazine (10D), 10-[6'-[N-Bis(hydroxyethylamino)hexyl]-2-chloro phenoxazine (11D), 10-[6'-[N-Morpholino]hexyl]-2-chlorophenoxazine (12D), 10-[6'-[N-Piperidino]hexyl]-2-chlorophenoxazine (13D), 10-[6'-[N-Pyrrolidino]hexyl]- 2-chlorophenoxazine (14D), 10-[6'-[N-(β-hydroxyethyl) piperazino]-hexyl-2-chlorophenoxazine (15D) and 10-[6'-[N- Thiomorpholino]hexyl]-2-chlorophenoxazine (16D), were studied by the well-diffusion method. Bacteria such as Klebsialla sp., Bacillus sp., Proteus vulgaris, Salmonella sp., Shigella flexneri, Staphylococcus aureus, Streptococcus pneumoniae, and Vibrio cholerae were used to investigate the antibacterial activities. No antibacterial activity was observed with 12D and 16D while 15D exhibited antibacterial activity against a few tested bacteria. All other compounds (10D, 11D, 13D and 14D) studied exhibited significant antibacterial activities against all the bacteria screened.

Keywords: Phenoxazine derivatives, antibacterial activity, well-diffusion method

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INTRODUCTION

Pharmaceutical companies have been producing a number of new antibacterials in the last years; however, resistance to these drugs has increased and has now become a global concern [1]. The global emergence of multi-drug resistant (MDR) bacteria is limiting the effectiveness of current drugs and significantly causing treatment failure [2]. Due to the increase of resistance to antibiotics, there is a pressing need to develop new and innovative antimicrobial agents.

Heterocyclic compounds continue to attract considerable interest due to their diverse biological activities. Phenoxazines are a group of N-heterocyclic compounds having three ring structures with nitrogen and oxygen atoms [3]. Phenoxazines and related compounds have shown diverse biological activities such as tranquilizers [4], anti-inflammatory [5], antimalarial [6], antipsychotropic [7], antiviral [8], antitubercular [9, 10], antitumour [11, 12], stimulation of the penetration of anticancer agents via the blood-brain barrier [13] and multidrug resistance reversal activity [14]. They have also been found to prevent human amyloid disorders [15] and to protect neuronal cells from

death by oxidative stress [16]. They bind to physiological targets or receptors, producing many possible mechanisms of actions.

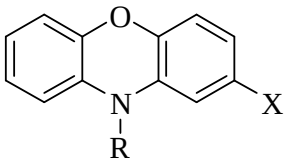
A slight variation in the substitution pattern on the phenoxazine nucleus often causes a marked difference in activities and therefore phenoxazines with various substituents are being synthesized and tested for activities in search of better medicinal agents. Thimmaiah et al. [17-21] have reported the chemistry and biology of a number of N^{10}-substituted phenoxazines synthesized originally as modulators of P-glycoprotein-mediated multidrug-resistance (MDR). It has been reported [22] that some phenoxazines inhibit intracellular replication of viruses including human immunodeficiency viruses (HIV). Furthermore, some of these derivatives have been reported to exhibit significant anticancer activities [23, 24] and antimicrobial activities including antibacterial activities [25, 26]. Owing to this great interest has arisen in the design and synthesis of new phenoxazines to explore their biological activities. In the present investigations hexyl series compounds of phenoxazine have been studied for their antibacterial activity.

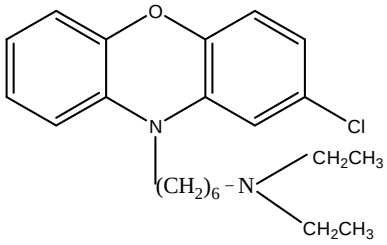
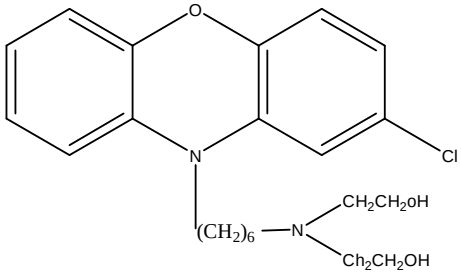
EXPERIMENTAL SECTION

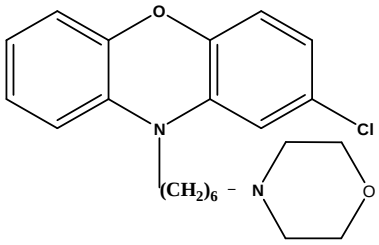
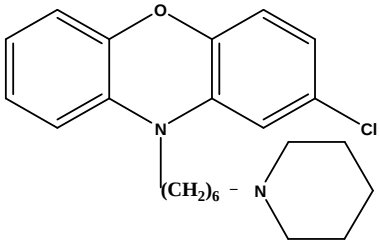
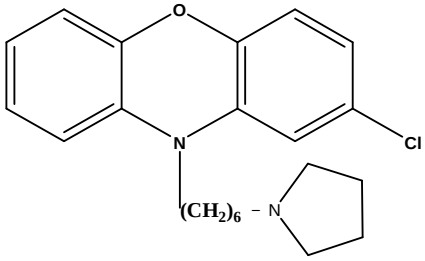
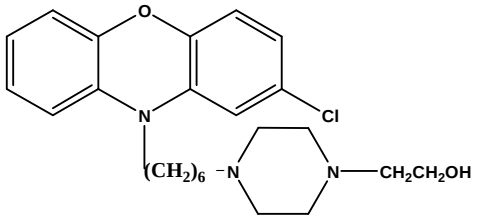
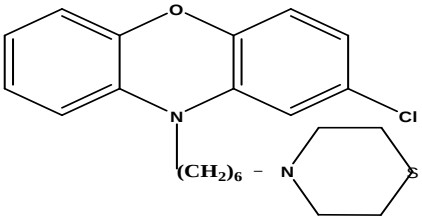
Phenoxazine derivatives

Hexyl series compounds of phenoxazine 10-[6'-[N-Diethylamino]hexyl]-2-chlorophenoxazine (**10D**), 10-[6'-[N-Bis(hydroxyl ethylamino)hexyl]-2-chlorophenoxazine (**11D**), 10-[6'-[N-Morpholino]hexyl]-2-chlorophenoxazine (**12D**), 10-[6'-[N-Piperidino]hexyl]-2-chloro-phenoxazine (**13D**), 10-[6'-[N-Pyrrolidino]hexyl]- 2-chlorophenoxazine (**14D**), 10-[6'-[N-(β-hydroxyethyl) piperazino]-hexyl-2-chlorophenoxazine (**15D**) and 10-[6'-[N-Thiomorpholino]hexyl]-2-chlorophenoxazine (**16D**), were studied. The synthesis procedures of these compounds are presented else where. The structures of these compounds are presented in Table 1.

Table-1: Structures of the phenoxazine and its derivatives used in the present studies



Sl. No.	Compounds	Structure
1.	10-[6'-[N-Diethylamino]hexyl]-2-chloro-phenoxazine (10D)	
2.	10-[6'-[N-Bis(hydroxyethylamino)hexyl]-2-chloro-phenoxazine (11D)	

3.	10-[6'-[N-Morpholino]hexyl]-2-chloro-phenoxazine (12D)	
4.	10-[6'-[N-Piperidino]hexyl]-2-chloro-phenoxazine (13D)	
5.	10-[6'-[N-Pyrrolidino]hexyl]- 2-chloro-phenoxazine (14D)	
6.	10-[6'-[N-(b-hydroxyethyl)piperazino]-hexyl-2-chloro-phenoxazine (15D)	
7.	10-[6'-[N- Trimorpholino]hexyl]-2-chloro-phenoxazine (16D)	

Antibacterial activity assay

The pure cultures of human pathogenic bacteria such as *Klebsialla sp.*, *Bacillus sp.*, *Proteus vulgaris*, *Salmonella sp.*, *Shigella flexneri*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Vibrio cholerae*, were obtained from the culture collection centre, Department of Microbiology, J.S.S. Medical College, Mysore, India. The well-diffusion method was used to screen the antibacterial activity using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were inoculated with 1 x 10^5 cfu cultures of test bacteria. Agar cup of 5mm diameter were made in the plates. 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml, 2.5 mg/ml solutions of each derivative were prepared using distilled water. 50µ1 volume of each aqueous extract was introduced into wells in MHA plates already seeded with the standardized inoculums (1x10^5 cfu/ml) of the test bacterial cells. 50µ1 of distilled water was introduced to one of the well as control. The compound was allowed to diffuse for 5 minutes and

the plates were kept for incubation at 37°C for 24 h. After incubation, inhibition zones formed around the wells were measured with transparent ruler. Triplicates were maintained and the experiments were conducted twice.

RESULTS

Antibacterial activity of phenoxazine derivatives against different human pathogenic bacteria is presented in Tables 2 - 6. The results of antibacterial studies of newly synthesized compounds reveal that the compounds possess significant antibacterial activities. Among the compounds tested, 12D and 16D did not exhibit any antibacterial activity even at the maximum tested concentration of 2.5mg/ml. Among those that exhibited antibacterial activity 10D exhibited superior antibacterial activity followed by 11D, 14D, 13D and 15D.

10-[6'-[N-Diethylamino]hexyl]-2-chlorophenoxazine (10D) exhibited good antibacterial activity against the tested bacterial strains at 0.5mg/ml, except *S. pneumoniae* wherein antibacterial activity was observed at 2.0mg/ml and above. Best activity was observed against *S. aureus* followed by *S. flexneri* and almost equal activity was observed against *V. cholerae*, *P. vulgaris* and *Klebsiella sp.* (Table 2). Compounds 11D, 14D and 13D showed antibacterial activity against all the tested bacteria at 0.5mg/ml concentration though the degree of activity varied with respect to the type of bacteria. 10-[6'-[N-Bis(hydroxyethylamino)hexyl]-2-chlorophenoxazine (11D) exhibited highest activity against *S. flexneri* followed by *Salmonella* sp., *S. aureus*, *Bacillus* sp. and least activity was observed against *S. pneumonia* (Table 3). 10-[6'-[N-Piperidino]hexyl]-2-chlorophenoxazine (13D) showed comparatively better activity against *Salmonella* sp. and *S. aureus*, while least activity was observed against *Klebsiella* sp (Table 4). With respect to 10-[6'-[N-Pyrrolidino]hexyl]- 2-chlorophenoxazine (14D), best antibacterial activity was observed against *P. vulgaris* followed by *Salmonella* sp. and *V. cholerae*. Least activity was observed against *S. aureus* (Table 5). Out of five compounds tested, 10-[6'-[N-(β-hydroxyethyl)piperazino]-hexyl-2-chlorophenoxazine (15D) was the least active. This compound did not exhibit any antibacterial activity against *Klebsiella* sp., *P. vulgaris*, *S. aureus* and *V. cholerae* even at the highest concentration tested of 2.5mg/ml (Table 6). It showed reasonably good activity against *Bacillus* sp. and *Salmonella* sp.

In general, the activity was dose dependent and varied with the type of compound and the organism. The compounds exhibited similar level of antibacterial activity against both Gram-negative and Gram-positive bacteria.

Table-2: Antibacterial activity 10-[6'-[N-Diethylamino]hexyl]- 2-chloro-phenoxazine (10D) against some human pathogenic bacteria at different concentrations

Sl. No.	Concentrations	Human pathogenic bacteria [Zone of inhibition (mm)]							
		***Bacillus* sp.**	***Klebsiella* sp.**	***Proteus vulgaris***	***Salmonella* sp.**	***Shigella flexneri***	***Staphylococcus aureus***	***Streptococcus pneumoniae***	***Vibrio cholerae***
1.	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.	0.5mg/ml	11.0	11.0	12.75	13.75	16.0	16.0	0.0	14.5
3.	1.0mg/ml	11.75	13.0	13.25	15.25	18.75	18.5	0.0	20.5
4.	1.5mg/ml	17.5	15.5	21.5	16.75	20.75	21.0	0.0	21.5
5.	2.0mg/ml	18.0	18.75	22.0	18.75	22.0	24.5	11.0	22.0
6.	2.5mg/ml	19.25	21.25	22.25	19.5	24.75	25.0	11.75	22.5

Values are means of two experiments and each with three replicates

Table-3: Antibacterial activity 10-[6'-[N-Bis(hydroxyethylamino)hexyl]- 2-chloro-phenoxazine (11D) against some human pathogenic bacteria at different concentrations

Sl. No.	Concentrations	Human pathogenic bacteria [Zone of inhibition (mm)]							
		***Bacillus* sp.**	***Klebsiella* sp.**	***Proteus vulgaris***	***Salmonella* sp.**	***Shigella flexneri***	***Staphylococcus aureus***	***Streptococcus pneumoniae***	***Vibrio cholerae***
1.	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.	0.5mg/ml	11.75	14.0	10.25	14.25	12.25	14.25	11.25	13.75
3.	1.0mg/ml	12.75	14.5	11.25	17.25	13.25	14.75	11.75	14.75
4.	1.5mg/ml	14.75	15.5	14.25	18.5	15.25	17.75	13.5	15.75
5.	2.0mg/ml	16.25	16.0	14.75	19.5	17.75	18.25	13.75	16.25
6.	2.5mg/ml	19.75	17.0	18.25	21.5	23.0	21.25	14.0	16.75

Values are means of two experiments and each with three replicates

Table-4: Antibacterial activity 10-[6'-[N-Piperidino]hexyl]- 2-chloro-phenoxazine (13D) against some human pathogenic bacteria at different concentrations

Sl. No.	Concentrations	Human pathogenic bacteria [Zone of inhibition (mm)]							
		***Bacillus* sp.**	***Klebsiella* sp.**	***Proteus vulgaris***	***Salmonella* sp.**	***Shigella flexneri***	***Staphylococcus aureus***	***Streptococcus pneumoniae***	***Vibrio cholerae***
1.	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.	0.5mg/ml	9.75	10.5	12.0	12.75	9.5	14.0	10.0	10.75
3.	1.0mg/ml	10.25	11.0	14.25	17.25	11.25	14.5	12.0	12.0
4.	1.5mg/ml	11.25	11.75	15.25	19.75	12.0	17.0	13.5	13.5
5.	2.0mg/ml	12.25	12.25	16.25	20.5	12.25	17.75	14.25	14.5
6.	2.5mg/ml	13.75	13.25	17.25	22.0	13.75	20.25	15.0	17.0

Values are means of two experiments and each with three replicates

Table-5: Antibacterial activity 10-[6'-[N-Pyrrolidino]hexyl]- 2-chloro-phenoxazine (14D) against some human pathogenic bacteria at different concentrations

Sl. No.	Concentrations	Human pathogenic bacteria [Zone of inhibition (mm)]							
		***Bacillus* sp.**	***Klebsiella* sp.**	***Proteus vulgaris***	***Salmonella* sp.**	***Shigella flexneri***	***Staphylococcus aureus***	***Streptococcus pneumoniae***	***Vibrio cholerae***
1.	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.	0.5mg/ml	10.75	15.25	10.75	10.75	16.0	15.75	11.0	13.25
3.	1.0mg/ml	11.75	17.0	15.0	17.75	16.25	17.75	13.0	16.75
4.	1.5mg/ml	15.0	17.5	16.75	18.0	18.25	19.25	15.5	18.25
5.	2.0mg/ml	19.25	19.75	22.25	21.0	19.25	19.5	17.75	21.5
6.	2.5mg/ml	20.25	21.5	24.5	23.0	20.0	18.5	21.75	22.75

Values are means of two experiments and each with three replicates

Table-6: Antibacterial activity 10-[6'-[N-(b-hydroxyethyl)piperazino]-hexyl-2-chloro-phenoxazine (15D) against some human pathogenic bacteria at different concentrations

Sl. No.	Concentrations	Human pathogenic bacteria [Zone of inhibition (mm)]							
		***Bacillus* sp.**	***Klebsiella* sp.**	***Proteus vulgaris***	***Salmonella* sp.**	***Shigella flexneri***	***Staphylococcus aureus***	***Streptococcus pneumoniae***	***Vibrio cholerae***
1.	Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.	0.5mg/ml	11.0	0.0	0.0	11.75	11.25	0.0	9.5	0.0
3.	1.0mg/ml	15.0	0.0	0.0	12.0	14.75	0.0	12.0	10.0
4.	1.5mg/ml	17.5	0.0	0.0	12.75	15.0	0.0	12.75	12.0
5.	2.0mg/ml	18.25	0.0	0.0	13.0	16.25	0.0	13.0	13.5
6.	2.5mg/ml	19.5	0.0	0.0	13.75	17.0	0.0	13.5	14.0

Values are means of two experiments and each with three replicates

DISCUSSION

Phenoxazine derivatives are known to have biological activities including antibacterial activity. In the present work, antibacterial activity of seven phenoxazine derivatives was studied. Out of seven phenoxazine derivatives tested five derivatives exhibited significant antibacterial activity. Similar antibacterial activity of a few other phenoxazine derivatives have been reported [25-32].

The antibacterial antibiotics and alkaloids produced by actinomycetes are known to possess phenoxazine skeleton in them [32, 33]. This indicates that the phenoxazine parent ring is essential for antibacterial characteristics. Phenoxazine derivative was reported to cause severe morphological changes such as membrane blebbing and formation of hollows in *Helicobacter pylori*, in addition to induction of heat shock protein 60. All these features seem to contribute for antibacterial activity against *H. pylori* [26]. Phenoxazine derivatives were reported to interchalate with DNA base pairs resulting in DNA loss [34, 35] leading to their biological activity.

Ability of drugs to bind to plasma protein when they enter into blood plasma system of organism is a fundamental factor in determining the overall pharmacological activity of a drug. Such drug-protein complexes alter elimination of drug from metabolism and thereby prolonging the exposure to drugs. The drug-albumin interaction is analyzed *in vitro* by their ability to bind with bovine serum albumin. The phenoxazine derivatives were reported to exhibit significant binding properties with bovine serum albumin [19-21] which make them good candidates for human disease treatment.

CONCLUSION

The hexyl phenoxazine derivatives presented significant antibacterial activity *in vitro*, arising as good candidates for further studies.

REFERENCES

[1] G Adwan; M Mhanna, *Journal of Scientific Research*, **2008**, 3, 134–139.
[2] RE Hancock, *Lancet Infect. Dis*., **2005**, 5, 209–218.
[3] N Motohashi; LA Mitscher; R. Meyer, *Med. Res. Rev*., **1991**, 11, 239-294.
[4] MK El-Said, *Pharmazie*, **1981**, 36, 678-679.
[5] SR Tilak; R Tyagi; B Goel; KK Saxena, *Indian drugs*, **1998**, 35, 216-221.
[6] JN Dominguez; S Lopez; J Charris; L Iarruso; G Lobo; A Semenow; JE Olson; PJ Rosenthal, *J. Med. Chem.*, **1997**, 40, 2726-2733.
[7] G Lin; KK Midha; EM Hawes, *J. Heterocycl. Chem*., **1991**, 28, 215-219.
[8] A Iwata; T Yamaguchi; K Sato; R Izumi; A Tomoda; *Tohoku. J. Exp. Med*., **2003**, 200, 161-165.
[9] M Viveros; L Amaral, *Int. J. Antimicrob. Ag*., **2001**, 17, 225-228.
[10] L Amaral; JE Kristiansen. *Int. J. Antimicrob. Ag*., **2000**, 14, 173-176.
[11] Motohasho , N.; Kawase, M.; Saito, S.; Sakagami, H. Curr. Drug Targets 2000, 1, 237.
[12] K Hara; M Okamoto; T Aki; H Yagita; H Tanaka; Y Mizukami; H Nakamura; A Tomoda; N Hamasaki; D Kang, *Mol. Cancer Ther*., **2005**, 4, 1121-1127.
[13] T Kurihara; N Motohasho; GL Pang; M Higano; K Kiguchi; J Molnar, *Anticancer Res*., **1996**, 16, 2757-2765.
[14] O Wesolowska; J Molnar; G Westman; K Samuelsson; M Kawase; I Ocsovszki; N Motohashi; K Michalak, *In Vivo*, **2006**, 20, 109-114.
[15] T Klabunde; HM Petrassi; VB Oza; P Raman; JW Kelly; JC Sacchettini, *Nat. Struct. Biol*., **2000**, 7, 312-321.
[16] B Moosmann; T Skutella; K Beyer; C Behl, *Biol. Chem*., **2001**, 382, 1601-1612.
[17] KN Thimmaiah; BS Jayashree; GS Germain; PJ Houghton; JK Horton, *Oncol. Res.*, **1998**, 10: 29-41.
[18] KN Thimmaiah; JB Easton; GS Germain; CL Morton; S Kamath; JK Buolamwini; PJ Houghton, *J. Biol. Chem*., **2005**, 280: 31924-31935.
[19] BC Channu; HN Kalpana; GB Eregowda; C Dass; PJ Houghton; KN Thimmaiah, *J. Pharm. Biomed. Anal.*, **1999**, 21: 775-785.
[20] GB Eregowda; BC Channu; S Jagadeesh; HN Kalpana; R Hegde; PJ Houghton; KN Thimmaiah, *Indian Journal of Chemistry- Section B*, **2000**, 39B: 680-687.
[21] Kalpana, H. N.; Channu, B. C.; Chhabil Dass, Houghton, P. J.; Thimmaiah, K. N. Journal of Chemical Sciences, **2000**, 112(1): 51-61.
[22] RA Floyd; JE Scheider; YQ Zhu; TW North; F Schinazi, *Proc. Am. Assoc. Cancer. Res*., **1993**, 34, 359.
[23] T Kurihara; N Motohashi; HH Sakagami; J Molnar, *Anticancer Res*., **1999**, 19, 4081-4083.
[24] T Kurihara; K Nojima; H Sakagami; N Motohashi; J. Molnar, *Anticancer Res*., **1999**, 19, 3895-3899.
[25] S Shimizu; M Suzuki; A Tomoda; S Arai; H Taguchi; T Hanawa; S Kamiya, *The Tohoku Journal of Experimental Medicine*, **2004**, 203, 47-52.
[26] T Hanawa; T Osaki; T Manzoku; M Fukuda; H Kawakami; A Tomoda; S Kamiya, *Biol. Pharm. Bull.*, **2010**, 33, 188-191.
[27] Hidayat Hussain; Sabine Specht; Salem R. Sarite; Michael Saeftel; Achim Hoerauf; Barbara Schulz; Karsten Krohn, *J. Med. Chem.*, **2011**, 54, 4913–4917.
[28] KG Ojha; SP Mathur; AK Mathur; AS Chittoria; H Tahiliani; RK Sharma, *Indian Journal of Chemistry*, **2004**, 43, 2254-2256.
[29] K Pandurangan; S Gallagher; GG Morgan; H Müller-Bunz; F Paradisi, *Metallomics*, **2010**, 8, 530-534.
[30] Tomoko Hanawa; Takako Osaki; Taki Manzoku; Minoru Fukuda; Hayato Kawakami; Akio Tomoda; Shigeru Kamiya, *Biological and Pharmaceutical Bulletin*, **2010**, 33, 188-191.
[31] http://www.lens.org/images/patent/US/4689325, **1987**.
[32] M Kiran Kumar; K Sunitha; K Bhupathi Reddy; M Jayanth Kumar; NB Shobha Rani; G Mohan Rao; B Vijaya Kumar, *World Journal Of Pharmacy and Pharmaceutical Sciences*, **2013**, 2, 5284-5295.
[33] J Ren; D Liu; L Tian; Y Wei; P Proksch; J Zeng; W Lin, *Bioorganic and Medicinal Chemistry Letters*, **2013**, 23: 301-304.
[34] N Motohashi; Yakugaku Zasshi, *Journal of the Pharmaceutical Society of Japan*, **1982**, 102, 646-650.
[35] KH Chandramouli; C D'Souza; KN Thimmaiah, *Nucleosides, Nucleotides and Nucleic Acids*, **2008**, 27: 70-83.