Synthesis, Antimicrobial Evaluations, and DNA Photo Cleavage Studies of New Bispyranopyrazoles

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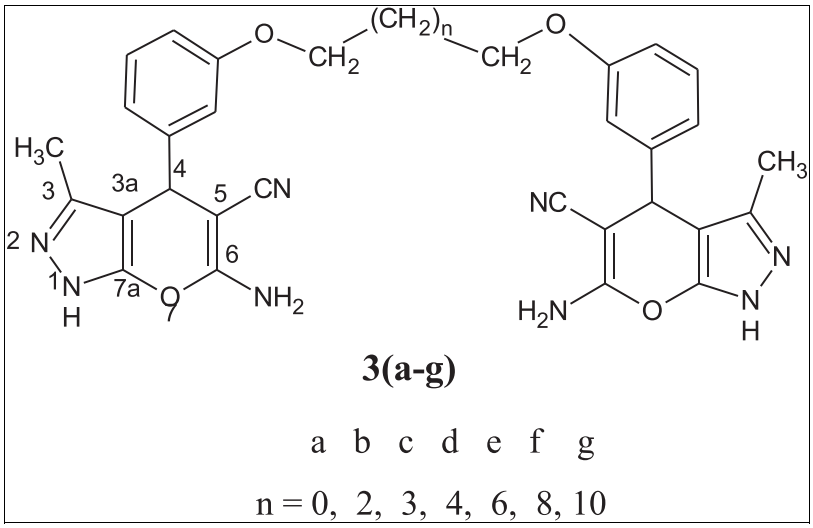


3(a-g)

a b c d e f g

n = 0, 2, 3, 4, 6, 8, 10

The bispyranopyrazoles **4(a–g)** were synthesized from the reactions of dibenzaldehydes **2(a–g)** with 3-methylpyrazole-5-one **3** and malononitrile by refluxing under alcoholic medium. The dibenzaldehydes were obtained from the O-alkylation of 3-hydroxybenzaldehyde with suitable 1,ω-dibromoalkanes under the alkaline conditions in the presence of dry EtOH/DMF. The structures of bisheterocyclics were determined from rigorous analysis of their spectral parameters (IR, ^{1}H-NMR, ^{13}C-NMR, and ESI-MS). The newly prepared compounds were screened for their antimicrobial activity against seven bacterial and five fungal strains. The DNA photo cleavage potential of these compounds was also evaluated by using agarose gel electrophoresis, and bispyranopyrazoles **4b, 4d,** and **4e** exhibited significant level of DNA photocleavage activity.

INTRODUCTION

Heterocyclic chemistry is an integral part of the chemical sciences and constitutes a considerable part of the modern researches that are occurring presently throughout the world. A wide range of heterocyclic compounds have been synthesized in order to develop physiologically and pharmacologically active products. Pyranopyrazoles are the useful heterocycles where pyran and pyrazole moiety coexist in the same structure, and these substances have been prepared extensively in the past because of their various biological properties [1–3]. Synthesis of these compounds has attracted the attentions of organic chemists in the last decades on account of their prominent utilization as analgesic, anti-inflammatory, ulcerogenic [4], antibacterial, antifungal [5,6], antitubercular [7], antimalarial [8], antitumor [5,9,10], antioxidant [10], antiproliferative [11], antihypertensive [12], hypnotic [13], and vasodilator [14]. Recently, some pyrazole derivatives are reported to have moderate anticancer activity [15], and some of their derivatives are also found to have broad spectrum biological activities [16–25]. Substituted pyrano[2,3-*c*]pyrazoles have been found to be effective antiplatelet molecules [26] which affect K^+ induced calcium dependent aortal contraction. Several pyrano[2,3-*c*]pyrazol-4-ones have demonstrated an affinity toward A1 and A2a adenosine receptors [27]. Also 6-amino-5-cyano-dihydropyranopyrazoles have been identified as a screening hit for human Chk1 kinase inhibitors [28].

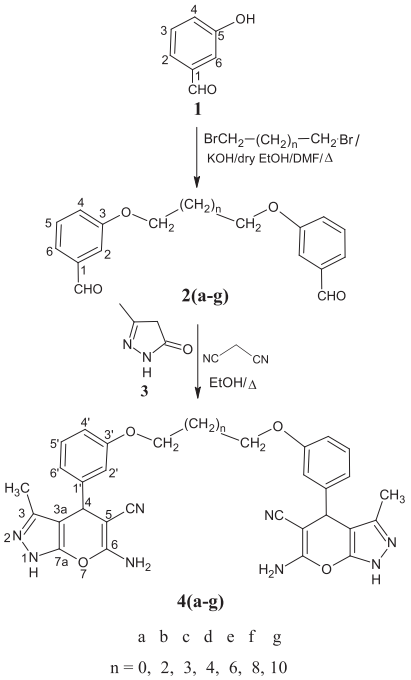
In recent years, attention has also been paid to evaluate the DNA photocleavage potential of some azoles [29–31] and heteroaryl-linked hydrazones [32] because of their binding or interacting ability with the DNA structure. DNA is a site where most of the chemotherapeutic drugs act and interact which may result in DNA photocleavage leading to inhibition of cancerous cells [33]. Therefore, these nitrogen containing heterocyclic compounds could

be used as probes for DNA structure, and potential chemotherapeutic and diagnostic agents [34].

Dihydropyrano[2,3-*c*]pyrazole was first synthesized by a reaction between 3-methyl-1-phenylpyrazolin-5-one and tetracyanoethylene [35]. H. Otto and co-workers reported a base catalyzed two-component Michael type reaction between 4-arylidiene-1-phenyl-1*H*-pyrazol-5-one and malononitrile for the synthesis of various 4-aryl-4*H*pyrano[2,3-*c*] pyrazoles [36]. The most common and convenient approach toward diverse pyranopyrazoles is a base catalyzed three-component reaction [37] of pyrazol-5-one, aldehydes, and malononitrile.

Because of the importance of pyranopyrazole derivatives considerable efforts have been made by several investigators, to prepare new compounds bearing a single substituent or more complicated systems, but the synthesis of the compounds containing two pyranopyrazoles in the same molecule has not been much explored in the literature. Only one paper regarding the synthesis of two bispyranopyran molecules was found [38]. Motivated by these results, this protocol was evaluated for its suitability to build new bispyranopyrazole scaffolds which were further investigated for their DNA photocleavage study. These compounds are formed by joining two pyranopyrazole moieties together through the aliphatic carbon chains of varying lengths. By keeping this aspect in view and in continuation to our studies on the bisheterocycles [39–41], the present researches are focussed upon the transformations of dibenzaldehydes **2(a–g)** to bispyranopyrazoles **4(a–g)** built around the alkyl chains consisting of two to twelve methylene groups. The major interest behind study was to investigate the effect of the central spacer length upon the formation and antimicrobial behavior of the bispyranopyrazoles **4(a–g)**.

Scheme 1. Synthesis of bispyranopyrazoles.



RESULTS AND DISCUSSION

The bispyranopyrazoles **4(a–g)** needed for the present investigations were obtained starting from 3-hydroxybenzaldehyde **1** which was reacted with suitable 1,ω-dibromoalkanes (1,2-dibromoethane, 1,4-dibromobutane, 1,5-dibromopropane, 1,6-dibromohexane, 1,8-dibromooctane, 1,10-dibromodecane, and 1,12-dibromododecane) under refluxing conditions in the presence of dry EtOH/KOH/DMF to yield dibenzaldehydes **2(a–g)** [42]. The later were treated with malononitrile and 3-methylpyrazole-5-one **3** [43,44] to furnish bispyranopyrazole**s 4(a–g)** in good yields (Scheme 1).

The structures of the newly prepared compounds were elucidated on the basis of their spectroscopic data (IR, ^{1}H-NMR, ^{13}C-NMR, and ESI-MS), and elemental analysis results also confirmed the purity of these products.

IR spectra of dibenzaldehydes **2(a–g)** showed strong absorption bands at 1698–1679 cm^{-1} because of conjugated C=O group and two additional bands at 2874–2850 and 2759–2717 cm^{-1} appeared because of aldehyde C—H stretching.

In the ^{1}H-NMR spectra (400 MHz, DMSO-d_6) of dibenzaldehydes **2(a–g)**, the downfield signal at δ 9.98–9.89 could be ascribed to CHO proton appeared. The hydrogens (H-2, 6, 5, and 4) of the phenyl ring were found to be placed at d 7.86–7.37 (2H, s), 7.49–7.36 (2H, m), 7.46–7.30 (2H, d, J_o = 6.6 Hz), and 7.27–7.09 (2H, m) respectively. The resonances of internal chain O***C*H*$_2$** group were placed in the aliphatic region at δ 4.45–3.93.

^{13}C-NMR (100 MHz, DMSO-d_6) spectra of dibenzaldehydes **2(a–g)** proved instrumental to corroborate their proposed structures. The presence of aldehyde group was confirmed by appearance of signal at δ 192.37–192.12 (C=O). The aromatic carbon atoms showed suitable signals at δ 159.72–159.13 (C-3), 137.80–137.51 (C-1), 130.08–129.98 (C-6), 123.56–122.20 (C-5), 122.00–121.16 (C-4), and 113.35–112.59 (C-2); the downfield resonance of C-3 as compared to other carbon atoms can be ascribed to its direct linkage to the electronegative oxygen atom. The resonance placed at δ 68.30–67.60 could be generated by internal chain O***C***H$_2$ group.

IR spectra of **4(a–g)** were very helpful to corroborate their structures which did not exhibit any absorption at 1698–1679 cm^{-1} indicating the involvement of carbonyl group during the formation of bispyranopyrazoles **4(a–g)**. In spite of this, a significant absorption bands were observed at 3392–3350, 3228–3207 (NH_2), 3158–3138 (NH), 2958–2933,

2888–2844 (methylene C—H), 2258–2250 (CN), 1606–1595 (C=N), and 1252–1249, 1088–1032 cm^{-1} (C—O).

A comparison of the ^{1}H-NMR spectra (400 MHz, DMSO-d_6) of **2(a–g)** and **4(a–g)** showed that resonances present at δ 9.98–9.89 in former were found missing altogether in the later thereby pronouncing the involvement of these hydrogens in the chemical transformation. A broad singlet at δ 11.32–11.09 could be ascribed to because of pyrazole ring N***H*** protons. The significant feature of these spectra was the signals of the two pyran ring protons (H-4) which were clearly resonating as a sharp singlet at δ 5.31–5.03. Here, the aromatic ring protons H-6′, 5′, 4′, and 2′ were centered at δ 7.32–7.09 (2H, d), 7.22–7.02 (2H, t), 7.13–6.95 (2H, t), and 7.03–6.82 (2H, brs) whereas 6-NH_2 group protons appeared as a sharp singlet at δ 6.88–6.65. In the aliphatic region, a triplet were also observed at δ 4.24–3.80 which may be assigned to OCH_2 group of aliphatic linker whereas other methylene groups were located at δ 2.19–1.22 with appropriate multiplicities (*vide experimental*). The singlets were also observed at δ 1.92–1.77 which could be ascribed to C_3—CH_3 protons.

The carbon framework of the compounds **4(a–g)** were fully explained on the basis of their ^{13}C-NMR spectra (100 MHz, DMSO-d_6). The downfield signals of C-6 were resonating at δ 162.58–162.30 because of its direct bonding to the electronegative nitrogen and oxygen atom and also its presence in double bond. The carbon atoms C-3′ and C-3 directly connected to oxygen and nitrogen atom produced suitable resonances at δ 157.91–157.69 and 139.15–139.09 respectively. The carbon atoms associated to the pyran ring C-7a and C-3a were placed at δ 135.43–135.29 and 116.43–116.30, while other two carbon atoms C-5 and C-4 provided suitable signals at δ 55.77–57.60 and 35.63–35.49 respectively. Remaining aromatic carbon atoms were found to be placed at the appropriate position in the aromatic region (see Experimental). The signals placed in the upfield region at δ 65.95–64.39 and 9.69–9.54 were assignable to internal chain OCH_2 and C_3—CH_3 group respectively.

BIOLOGICAL EVALUATION

Antimicrobial activity. The synthesized compounds **2(a–g)** and **4(a–g)** were screened for their *in vitro* antibacterial and antifungal activity against seven bacterial and five fungal species namely *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Pseudomonas fluorescens (MTCC 103)*, *Streptococcus pyogenes (MTCC 442)* and *Aspergillus janus* (MTCC 2751), *Penicillium glabrum* (MTCC 4951), *Fusarium oxysporum (MTCC 2480)*, *Aspergillus sclerotiorum (MTCC 1008)*, and *Aspergillus niger (MTCC 281)* respectively. Minimum inhibitory concentrations (MICs) were determined by using serial dilution technique.

Amoxicillin and fluconazole were used as the standard drug as positive control while the DMSO was used as negative control. This control did not show any activity against the strains of micro-organisms used. Normal saline was used to make a suspension of spore of bacterial and fungal strain for lawning. A loopful of particular microbial strain was transferred to 10-mL saline to get a suspension of corresponding species. After this, 1-mL volume of nutrient broth was added to each tube successively, and all the tubes were seeded with the bacterial and fungal strains. The minimum inhibitory concentration (MIC-μg/mL) was determined by using different dilutions of the concerned compound. The lowest concentration required to arrest the growth of bacterial and fungal strains was regarded as minimum inhibitory concentration (MIC).

The results were compared with positive controls, the standard drug Amoxicillin and Fluconazole. Serial dilution of the test compounds previously dissolved in dimethyl sulfoxide (DMSO) were prepared to final concentrations of 128, 64, 32, 16, 8, 4, 2, and 1 μg/mL. All the bacteria strains were grown at 37°C for 24 h in nutrient broth, and fungi were grown in malt extract at 28°C for 72 h. Each test compound was dissolved in DMSO and MIC (minimum inhibitory concentration) thus obtained were compared with control. The observed minimum inhibitory concentration (MIC-μg/mL) values are given in Tables 1 and 2. The susceptibility of the bacteria and fungi to the test compounds was determined by the appearance of turbidity after the above said time period.

All the synthesized compounds **2(a–g)** and **4(a–g)**, were found to be potent antimicrobial agents (Tables 1 and 2) against all tested strains.

It is evident from Table 1 that the compounds **2(a–g)** shows significant biological activity against various bacterial and fungal strains at (MIC-8 μg/mL). The compound **2g** showed significant activity against the most of strains namely; *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* (bacteria strains) and *P. glabrum*, *F. oxysporum*, *A. sclerotiorum* (fungi strains), while **2f** displayed activity against bacterial strains *K. pneumoniae*, *P. fluorescens*, *B. subtilis* and *A. janus*, *A. niger*, *A. sclerotiorum* respectively. Further **2c**, **2d** were also active with MIC-8 μg/mL against the *A. janus and E. coli* respectively. Also, **2e** showed potent microbial activity against *K. pneumoniae* and *F. oxysporum.*

It is evident from Table 2 that the compounds **4a** showed significant biological activity against the bacterial and fungal strains namely *K. pneumoniae, A. janus*, *A. niger*, and *A. sclerotiorum* at MIC of 8 μg/mL. The compound **4b** displayed significant activity against *P. aeruginosa*, *S. pyogenes* (bacteria strain), and *P. glabrum* (fungi strain). The compound **4c** displayed significant activity against *E. coli*, *S. pyogenes*, and *A. niger*, *F. oxysporum* at MIC of 8 μg/mL. Compound **4d** and **4e** were found to most active

Table 1

MIC (μg/mL) data of dibenzaldehydes 2(a–g).

Compound no.	Gram (−ve) bacteria				Gram (+ve) bacteria			Fungi				
	E. coli	*K. pneumoniae*	*P. Aeruginosa*	*P. fluorescens*	*S. aureus*	*B. subtilis*	*S. pyogenes*	*A. janus*	*P. glabrum*	*A. niger*	*F. oxysporum*	*A. sclerotiorum*
2a	16	32	32	16	32	64	32	64	32	16	32	32
2b	32	32	32	16	16	32	64	16	32	16	32	64
2c	16	32	16	32	16	16	32	**8**	32	32	16	32
2d	**8**	16	16	16	32	16	16	16	16	16	16	16
2e	16	**8**	16	32	16	16	16	16	16	16	**8**	16
2f	16	16	**8**	**8**	16	**8**	16	**8**	16	**8**	16	**8**
2g	**8**	16	**8**	16	**8**	**8**	16	16	**8**	16	**8**	**8**
Amoxicillin	**4**	**4**	**4**	**4**	2	2	**4**	—	—	—	—	—
Fluconazole	—	—	—	—	—	—	—	2	2	2	2	2

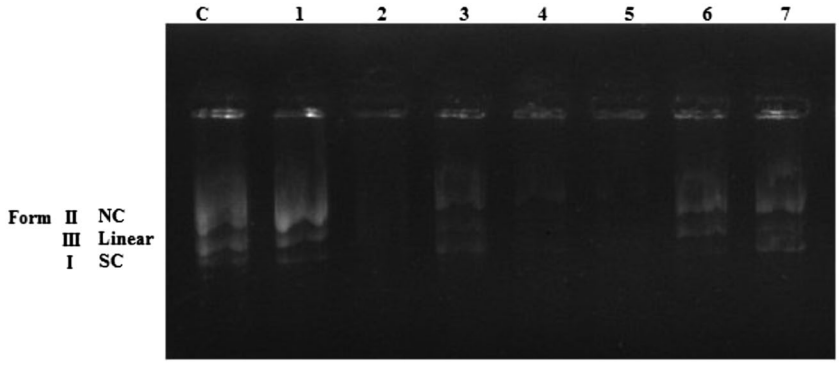
Bold emphasis signifies that concerned compounds showed MIC comparable to standard drugs against tested strains.

Table 2

MIC (μg/mL) data of bispyranopyrazoles 4(a–g)

Compound no.	Gram (−ve) bacteria				Gram (+ve) bacteria			Fungi				
	E. coli	*K. Pneumoniae*	*P. Aeruginosa*	*P. Fluorescens*	*S. aureus*	*B. Subtilis*	*S. Pyogenes*	*A. janus*	*P. glabrum*	*A. Niger*	F. oxysporum	A. sclerotiorum
4a	32	**8**	32	64	64	32	32	**8**	16	**8**	32	**8**
4b	64	32	**4**	16	32	**8**	16	32	**8**	16	32	16
4c	**8**	16	32	64	16	32	**8**	32	16	**8**	**8**	16
4d	**8**	32	**4**	64	32	32	64	16	16	**8**	32	**8**
4e	64	**8**	16	**8**	16	32	32	16	**8**	**8**	16	16
4f	64	32	**8**	32	**8**	32	**8**	**8**	**4**	16	**4**	16
4g	**4**	16	16	**8**	16	**8**	16	**4**	**8**	**4**	16	**8**
Amoxicillin	**4**	**4**	**4**	**4**	2	2	**4**	—	—	—	—	—
Fluconazole	—	—	—	—	—	—	—	2	2	2	2	2

Bold emphasis indicates that concerned compounds showed MIC comparable to standard drugs against tested strains.



	C	1	2	3	4	5	6	7
% NC	83.35	86.36	0.0	74.64	0.0	0.0	78.73	81.92
% Linear	12.16	11.88	0.0	15.73	0.0	0.0	21.26	11.15
% SC	3.72	1.38	0.0	9.62	0.0	0.0	0.0	6.91

Figure 1. DNA photo cleavage study of bispyranopyrazoles **4(a–g)**.

against *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *A. niger*, *A. sclerotiorum*, and *P. glabrum* with MIC of 8 and 4 μg/mL. Interestingly the compound **4f** showed MIC of 8 and 4 μg/mL against *P. aeruginosa*, *S. aureus*, *S. pyogenes* and *A. janus*, *P. glabrum* and *F. oxysporum*. Improved result has been observed in the case of **4g** which was found to inhibit the growth of bacterial strains *E. coli*, *P. fluorescens*, *B. subtilis* and fungal strains *A. janus*, *P. glabrum*, *A. niger*, *A. sclerotiorum* effectively at MIC of 4 and 8 μg/mL. This study reveals that some of synthesized compounds also showed MIC of 4 μg/mL against tested strains which is comparable to standard drug Amoxicillin.

DNA photocleavage study. DNA photocleavage experiment was performed by taking 10-μL solution containing pBR322 DNA in TE (*Tris* 10 mM, EDTA 0.01 mM, pH 8.0) buffer in the presence of 40 μg of synthesized compounds [45]. The sample solution held in caps of polyethylene micro centrifuge tubes was placed directly on the surface of a trans illuminator (8000 mW/cm) at 360 nm and was irradiated for 30 min at room temperature. After irradiation, samples were further incubated at 37°C for 1 h. Irradiated samples were mixed with 6× loading dye containing 0.25% bromophenol blue and 30% glycerol. The samples were then analyzed by electrophoresis on a 0.8% agarose horizontal slab gel in *Tris*–acetate EDTA buffer (40 mM *Tris*, 20 mM acetic acid, 1 mM EDTA, pH: 8.0). Untreated plasmid DNA was maintained as a control in each run of gel electrophoresis which was carried out at 5 V/cm for 2.0 h. Gel was stained with ethidium bromide (1 μg/mL) and photographed under UV light. To account the effect of synthesized compounds on DNA, the band intensities were analyzed using the GelQuant.NET software provided by biochemlabsolutions.com.

The DNA photo cleavage study was performed using agarose gel electrophoresis, and the overall pattern is shown in Figure 1.

However, compounds **4c, 4f** (Lane 4, 7) were responsible to decrease the intensity of Super coiled and Open circular forms of DNA.

No DNA cleavage was observed for negative control (lane C). A significant change in intensity of DNA Forms (I, II, and III) in case of synthesized compounds in comparison with untreated DNA indicated some kinds of fragmentations or interactions caused by the compounds. Decrease in intensity of plasmid DNA in case of compounds **4b**, **4c**, **4d**, **4e**, and **4f** (Lane 3, 4, 5, 6, and 7) as compared with control (Lane 1) indicated the cleavage of DNA forms. As shown in Figure 1, the compounds 4b, 4d, and 4e were responsible for the complete disappearance of all the forms (super coiled, open circular, and linear) of DNA.

The overall results observed from the present study have indicated that bispyranopyrazoles **4(a–g)** derivatives possessed significant potential for DNA photo cleavage study.

CONCLUSION

It may be concluded that this study describes the general and efficient method for the synthesis of new bispyranopyrazoles linked through the aliphatic chains under the ordinary conditions. It is evident from *in vitro* antimicrobial evaluations that prepared compounds showed marked potency against antimicrobial agents as compared to their intermediates bisaldehydes **2(a–g)** Further, DNA photo cleavage study reveals that compounds **4b**, **4d,** and **4e** have emerged as the most active DNA photo cleaving agents among all the synthesized compounds and some modifications in the basic structure may lead to construct some potential chemotherapeutic agents in future.

EXPERIMENTAL

All the chemicals used in this study were purchased from E. Merck, S. D. Fine Chem. Ltd., Mumbai and

Sigma-Aldrich. The melting points were determined by using the open capillary method and are uncorrected. The Infrared (IR) spectra were scanned in KBr pellets on a Perkin Elmer RXIFT Infrared spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in $CDCl_3$/DMSO-d_6 solvent on a 400-MHz Bruker spectrophotometer. The purity of the compounds was checked by TLC plates coated with silica gel (suspended in chloroform-methanol, 1:1), and iodine vapors were used as visualizing agent.

Synthesis of 3,3′-[alkane-1,n-diylbis (oxy)] dibenzaldehydes 2(a–g). 3-Hydroxybenzaldehyde **1** (0.01 mol) and KOH (0.01 mol) was dissolved in alcohol (100 mL), and then solvent was removed under vacuum. The residue was dissolved in DMF (25 mL) and 1,ω-dibromoalkanes (0.005 mol) was added slowly. The reaction mixtures were refluxed for 4 h, during which KBr was separated out. The solvent was removed under vacuum, and the remaining material was poured into iced HCl to give solid substances which were filtered under suction and thoroughly washed with water. The crude product thus obtained was crystallized from MeOH to yield pure compounds **2(a–g)**. The physical and spectral data of **2(a–g)** were found to be consistent as reported in literature [41].

Synthesis of 3-methyl-1H-pyrazol-5(4H)-one 3. Ethyl acetoacetate (0.5 mol) was taken in a 100-mL conical flask with absolute alcohol (20 mL), and then hydrazine hydrate (0.5 mol) was added drop wise with continuous shaking. The temperature of the reaction mixture was maintained throughout at 40°C. After the completion of reaction, a solid was separate which was filtered under suction and further crystallized from MeOH to yield pure 3-methylpyrazole-5-one as a white solid [42,43].

Synthesis of 4,4′-(3,3′-(ethane-1,2-diylbis(oxy))bis(3,1-phenylene))bis(6-amino-3-methyl-1,4-dihydropyrano[2–45]pyrazole-5-carbonitrile) 4a. A mixture of compound **2a** (0.01 mol), 3-methylpyrazole-5-one **3** (0.02 mol), and malononitrile (0.02 mol) was stirred in dry EtOH (25 mL) at 45°C for 3 h, and progress of reaction was monitored by TLC. The resulting reaction mixture was cooled to yield light yellow solid that was filtered, thoroughly washed with water and dried. The crude product thus obtained was crystallized from MeOH to yield a pure compound **4a**.

4a: Light yellow solid; Yield 72%; m.p.: 152–154°C; IR (KBr) cm^{-1} 3392 and 3210 (NH_2), 3158 (NH), 2956, 2888 (methylene C—H), 2259 (CN), 1606 (C=N), 1250, 1032 (C—O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.30 (2H, s, N***H***), 7.30 (2H, d, J = 7.1 Hz, H-6′), 7.21 (2H, t, J_o = 7.5 Hz, H-5′), 7.13 (2H, d, J_o = 7.4 Hz, H-4′), 7.03 (2H, s, H-2′), 6.88 (4H, s, N***H₂***), 5.31 (1H, s, H-4), 4.09 (4H, t, J_{vic} = 6.1 Hz, OC***H₂***), 1.92 (6H, s, C***H₃***); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.58 (C-6), 157.91 (C-3′), 139.15 (C-3), 136.15 (C-1′), 135.43 (C-7a), 129.30 (C-6′), 125.34 (C-5′), 117.30 (C≡N), 116.41 (C-3a), 113.47 (C-4′), 111.40 (C-2′). 65.95 (O***C***H_2), 57.77 (C-5), 35.63 (C-4), 9.71 (***C***H_3); ESI-MS: *m/z* 585 (M + Na, 10%), 563 (M + 1, 15%), 511 (10%), 489 (15%), 371 (9%), 294 (13%), 243 (19%), 242 (100%), 107 (8%), 93 (11%); *Anal.* Calcd. For $C_{30}H_{26}N_8O_4$: C, 64.05; H, 4.66; N, 19.92; found C, 64.09; H, 4.61; N, 19.96%.

Synthesis of 4,4′-(3,3′-(butane-1,4-diylbis(oxy))bis(3,1-phenylene))bis(6-amino-3-methyl-1,4-dihydropyrano[2–45]pyrazole-5-carbonitrile) 4b. The compound **4b** was synthesized from the reaction of **2b** (0.01 mol) with 3-methylpyrazole-5-one **3** (0.02 mol) and malononitrile (0.02 mol) under the same conditions as described earlier for **4a**.

4b: Yellow solid; Yield 74%; m.p.: 116–118°C; IR (KBr) cm^{-1} 3392 and 3207 (NH_2), 3138 (NH), 2953, 2850 (methylene C—H), 2256 (CN), 1600 (C=N), 1252, 1061 (C—O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.29 (2H, s, N***H***), 7.32 (2H, d, J = 7.1 Hz, H-6′), 7.22 (2H, t, J_o = 7.0 Hz, H-5′), 7.12 (2H, d, J_o = 7.5 Hz, H-4′), 7.00 (2H, brs, H-2′), 6.81 (4H, s, N***H₂***), 5.29 (1H, s, H-4), 4.24 (4H, t, J_{vic} = 6.3 Hz, OC***H₂***), 2.19 (2H, q, J_{vic} = 6.0 Hz, OCH_2C***H₂***), 1.81 (6H, s, C***H₃***); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.46 (C-6), 157.81 (C-3′), 139.11 (C-3), 136.11 (C-1′), 135.40 (C-7a), 129.28 (C-6′), 125.29 (C-5′), 117.26 (C≡N), 116.40 (C-3a), 113.33 (C-4′), 111.30 (C-2′). 64.42 (O***C***H_2), 57.70 (C-5), 35.54 (C-4), 29.23 (OCH_2***C***H_2), 9.67 (***C***H_3); ESI-MS: *m/z* 613 (M + Na, 28%), 591 (M + 1, 13%), 569 (15%), 543 (23%), 489 (16%), 301 (8%), 294 (21%), 242 (100%), 107 (9%), 94 (11%); *Anal.* Calcd. For $C_{32}H_{30}N_8O_4$: C, 65.07; H, 5.12; N,18.97; Found C, 65.11; H, 5.16; N, 18.93%.

Synthesis of 4,4′-(3,3′-(pentane-1,3-diylbis(oxy))bis(3,1-phenylene))bis(6-amino-3-methyl-1,4-dihydropyrano[2–45]pyrazole-5-carbonitrile) 4c. The compound **4c** was prepared from the reaction of **2c** (0.01 mol) with 3-methylpyrazole-5-one **3** (0.02 mol) and malononitrile (0.02 mol) under the same conditions as described earlier for **4a**.

4c: light yellow solid; Yield 73%; m.p.: 138–140°C; IR (KBr) cm^{-1} 3380 and 3219 (NH_2), 3147 (NH), 2958, 2870 (methylene C—H), 2254 (CN), 1598 (C=N), 1250, 1065 (C—O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.28 (2H, s, N***H***), 7.23 (2H, d, J = 7.0 Hz, H-6′), 7.19 (2H, t, J_o = 7.3 Hz, H-5′), 7.11 (2H, d, J_o = 7.6 Hz, H-4′), 7.01 (2H, brs, H-2′), 6.85 (4H, s, N***H₂***), 5.27 (1H, s, H-4), 4.08 (4H, t, J_{vic} = 6.4 Hz, OC***H₂***), 2.18 (4H, q, J_{vic} = 6.0 Hz, OCH_2C***H₂***), 1.98 (2H, m, OCH_2CH_2C***H₂***), 1.83 (6H, s, C***H₃***); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.49 (C-6), 157.85 (C-3′), 139.12 (C-3), 136.13 (C-1′), 135.41 (C-7a), 129.30 (C-6′), 125.32 (C-5′), 117.29 (C≡N), 116.43 (C-3a), 113.43 (C-4′), 111.39 (C-2′). 64.43 (O***C***H_2), 57.72 (C-5), 35.59 (C-4), 29.29 (OCH_2***C***H_2), 21.32 (OCH_2CH_2***C***H_2), 9.69 (***C***H_3); ESI-MS: *m/z* 627 (M + Na, 100%), 604 (M + 1, 3%), 590 (13%), 576 (9%), 548 (14%), 496 (5%), 386 (4%), 372 (7%), 344 (7%), 294 (11%), 238 (11%), 162 (13%), 121

(14%), 107 (11%), 94 (18%); *Anal.* Calcd. For $C_{31}H_{28}N_8O_4$: C, 64.57; H, 4.89; N,19.43; Found C, 64.62; H, 4.93; N, 19.39%.

Synthesis of 4,4′-(3,3′-(hexane-1,6-diylbis(oxy))bis(3,1-phenylene))bis(6-amino-3-methyl-1,4-dihydropyrano[2–45]pyrazole-5-carbonitrile) 4d. The compound **4d** was obtained by treating **2d** (0.01 mol) with 3-methylpyrazole-5-one **3** (0.02 mol) and malononitrile (0.02 mol) under the same conditions as used earlier for **4a**.

4d: Brown solid; Yield 80%; m.p.: 122–124°C; IR (KBr) cm^{-1} 3391 and 3214 (NH_2), 3140 (NH), 2935, 2865 (methylene C—H), 2257 (CN), 1595 (C=N), 1251, 1088 (C—O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.32 (2H, s, N***H***), 7.28 (2H, d, J_o = 7.4 Hz, H-6′), 7.19 (2H, t, J_o = 7.1 Hz, H-5′), 7.11 (2H, d, J_o = 7.2 Hz, H-4′), 6.93 (2H, brs, H-2′), 6.78 (4H, s, N***H₂***), 5.20 (1H, s, H-4), 4.19 (4H, t, J_{vic} = 6.6 Hz, OC***H₂***), 2.11 (4H, q, J_{vic} = 6.4 Hz, OCH_2CH_2), 1.92 (4H, m, $OCH_2CH_2CH_2$), 1.73 (6H, s, C***H₃***); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.46 (C-6), 157.80 (C-3′), 139.11 (C-3), 136.21 (C-1′), 135.40 (C-7a), 129.28 (C-6′), 125.29 (C-5′), 117.20 (C≡N), 116.38 (C-3a), 113.33 (C-4′), 111.30 (C-2′). 64.39 (OCH_2), 57.68 (C-5), 35.52 (C-4), 29.13 (OCH_2CH_2), 21.78 ($OCH_2CH_2CH_2$), 9.67 (CH_3); ESI-MS: *m/z* 641 (M + Na, 24%), 619 (M + 1, 26%), 590 (19%), 538 (12%), 493 (14%), 462 (11%), 398 (29%), 270 (14%), 242 (100%), 122 (21%), 108 (4%); *Anal.* Calcd. For $C_{34}H_{34}N_8O_4$: C, 66.01; H, 5.54; N,18.11; Found C, 65.97; H, 5.59; N, 18.15%.

Synthesis of 4,4′-(3,3′-(octane-1,8-diylbis(oxy))bis(3,1-phenylene))bis(6-amino-3-methyl-1,4-dihydropyrano[2–45]pyrazole-5-carbonitrile) 4e. The compound **4e** was prepared from the reaction of **2e** (0.01 mol) with 3-methylpyrazole-5-one **3** (0.02 mol) and malononitrile (0.02 mol) under the same conditions as described above for **3a**.

4e: Brown solid; Yield 65%, m.p.: 130–132°C; IR (KBr) cm^{-1} 3364 and 3210 (NH_2), 3145 (NH), 2938, 2867 (methylene C—H), 2250 (CN), 1598 (C=N), 1249, 1086 (C—O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.21 (2H, s, N***H***), 7.22 (2H, d, J_o = 7.8 Hz, H-6′), 7.13 (2H, t, J_o = 7.1 Hz, H-5′), 7.09 (2H, d, J_o = 7.0 Hz, H-4′), 6.92 (2H, s, H-2′), 6.81 (4H, s, N***H₂***), 5.29 (1H, s, H-4), 4.24 (4H, t, J_{vic} = 6.5 Hz, OC***H₂***), 1.86 (4H, quintet, J_{vic} = 6.5 Hz, OCH_2CH_2), 1.46 (4H, quintet, J_{vic} = 5.3 Hz, $OCH_2CH_2CH_2$), 1.35 (4H, m, $OCH_2CH_2CH_2CH_2$), 1.81 (6H, s, C***H₃***); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.30 (C-6), 157.72 (C-3′), 139.1 (C-3), 136.02 (C-1′), 135.29 (C-7a), 129.20 (C-6′), 125.18 (C-5′), 117.09 (C≡N), 116.33 (C-3a), 113.32 (C-4′), 111.23 (C-2′). 64.36 (OCH_2), 57.61 (C-5), 35.49 (C-4), 29.15 (OCH_2CH_2), 24.21 ($OCH_2CH_2CH_2$), 17.10 ($OCH_2CH_2CH_2CH_2$), 9.58 (CH_3); ESI-MS: *m/z* 668 (M + Na, 19%), 647 (M + 1, 16%), 642 (17%), 628 (13%), 586 (9%), 572 (12%), 516 (11%), 474 (14%), 473 (11%), 398 (29%), 214 (5%), 121 (10%), 108 (16%), 94 (12%); *Anal.* Calcd. For $C_{36}H_{38}N_8O_4$: C, 66.86; H, 5.92; N, 17.33; Found C, 66.90; H, 5.88; N, 17.37%.

Synthesis of 4,4′-(3,3′-(decane-1,10-diylbis(oxy))bis(3,1-phenylene))bis(6-amino-3-methyl-1,4-dihydropyrano[2–45]pyrazole-5-carbonitrile) 4f. The compound **4f** was synthesized from the reaction of **2f** (0.01 mol) with 3-methylpyrazole-5-one **3** (0.02 mol) and malononitrile (0.02 mol) under the same conditions as described earlier for **4a**.

4f: Light brown solid; Yield 76%; m.p.: 96–98°C. IR (KBr) cm^{-1} 3350 and 3222 (NH_2), 3149 (NH), 2933, 2844 (methylene C—H), 2253 (CN), 1599 (C=N), 1250, 1042 (C—O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.14 (2H, s, N***H***), 7.10 (2H, d, J_o = 7.5 Hz, H-6′), 7.04 (2H, t, J_o = 7.3 Hz, H-5′), 6.98 (2H, dd, J_o = 7.4, 2.3 Hz, H-4′), 6.86 (2H, brs, H-2′), 6.69 (4H, s, N***H₂***), 5.09 (1H, s, H-4), 4.13 (4H, t, J_{vic} = 5.9 Hz, OC***H₂***), 2.02 (2H, q, J_{vic} = 4.6 Hz, OCH_2CH_2), 1.79 (6H, s, C***H₃***), 1.51 (12H, m, $OCH_2CH_2CH_2CH_2CH_2$); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.41 (C-6), 157.72 (C-3′), 139.09 (C-3), 136.16 (C-1′), 135.35 (C-7a), 129.21 (C-6′), 125.26 (C-5′), 117.18 (C≡N), 116.40 (C-3a), 113.13 (C-4′), 111.25 (C-2′). 65.12 (OCH_2), 57.60 (C-5), 35.59 (C-4), 28.61 (OCH_2CH_2), 25.31 ($OCH_2CH_2CH_2$), 22.10 ($OCH_2CH_2CH_2CH_2$), 19.11 ($OCH_{2s}CH_2CH_2CH_2CH_2$); 9.54 (CH_3); ESI-MS: *m/z* 697 (M + Na, 100%), 675 (M + 1, 56%), 642 (17%), 584 (9%), 568 (12%), 493 (14%), 472 (11%), 398 (29%), 302 (14%), 214 (4%), 121 (21%), 93 (12%); *Anal.* Calcd. For $C_{38}H_{42}N_8O_4$: C, 67.64; H, 6.27; N, 16.61; Found C, 67.69; H, 6.31; N, 16.65%

Synthesis of 4,4′-(3,3′-(dodecane-1,12-diylbis(oxy))bis(3,1-phenylene))bis(6-amino-3-methyl-1,4-dihydropyrano[2–45]pyrazole-5-carbonitrile) 4g. The compound **4g** was obtained by reacting **2g** (0.01 mol) with 3-methylpyrazole-5-one **3** (0.02 mol) and malononitrile (0.02 mol) under the same conditions used earlier for **4a**.

4g: Grey solid; Yield 68%; m.p.: 104–106°C; IR (KBr) cm^{-1} 3359 and 3228 (NH_2), 3145 (NH), 2938, 2851 (methylene C—H), 2257 (CN), 1598 (C=N), 1252, 1044 (C—O); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.09 (2H, s, N*H*), 7.09 (2H, d, *J* = 7.0 Hz, H-6′), 7.02 (2H, t, J_o = 7.3 Hz, H-5′), 6.95 (2H, d, J_o = 7.6 Hz, H-4′), 6.82 (2H, brs, H-2′), 6.65 (4H, s, NH_2), 5.03 (1H, s, H-4), 3.80 (4H, t, J_{vic} = 6.1 Hz, OCH_2), 1.68 (2H, q, J_{vic} = 6.6 Hz, OCH_2CH_2), 1.77 (3H, s, CH_3), 1.22 (16H, m, $CH_2CH_2CH_2CH_2CH_2CH_2$); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 162.40 (C-6), 157.69 (C-3′), 139.08 (C-3), 136.10 (C-1′), 135.31 (C-7a), 129.19 (C-6′), 125.21 (C-5′), 117.15 (C≡N), 116.40 (C-3a), 113.11 (C-4′), 111.30 (C-2′). 64.42 (OCH_2), 57.70 (C-5), 35.54 (C-4), 2925.45 (OCH_2CH_2), 25.34 ($OCH_2CH_2CH_2$), 23.21 ($OCH_2CH_2CH_2CH_2$), 19.11 ($OCH_2CH_2CH_2CH_2CH_2$), 15.01 ($OCH_2CH_2CH_2CH_2CH_2CH_2$), 9.67 ($CH_3$); ESI-MS: *m/z* 725 (M + Na, 21%), 703 (M + 1, 13%), 629 (4%), 581 (9%), 527 (8%), 428 (5%), 372 (4%), 372 (7%), 313 (19%), 294 (15%), 239 (11%), 162 (13%). 107 (11%); *Anal.* Calcd. For $C_{40}H_{46}N_8O_4$: C, 68.35; H, 6.60; N, 15.94; Found C, 68.39; H, 6.56; N, 15.89%.

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REFERENCES AND NOTES

[1] El-Gazzar, A. B. A.; El-Enany, M. M.; Mahmoud, M. N. Bioorg Med Chem 2008, 16, 3261.

[2] Fayed, A. A.; Hosni, H. M; Flefel, E. M.; Amr, A. A. E. World J Chem 2009, 4. Mol 2012, 17, 10387.

[3] Sondhi, S. M.; Singh, N.; Johar; M.; Kumar, A. Bioorg Med Chem 2005, 13, 6158.

[4] Hafez, H. S.; Abbas, H. N.; El-Gazzar, A. B. A. Acta Pharm 2008, 58, 359.

[5] Fathalla, O. A.; Zeid, I. F.; Haiba, M. E.; Soliman, A. M.; Abd-Elmoez; S. I.; Serwy; W. S. World J Chem 2009, 4, 127.

[6] El-Assiery, S. A.; Sayed, G. H.; Fouda, A. Acta Pharm 2004, 54, 143.

[7] Vaghasiya, S. J.; Dodiya, D. K.; Trivedi, A. R.; Shah, V. H. ARKIVOC 2008, xii, 1.

[8] Klein, M.; Diner, P.; Dorin-Semblat, D.; Doerig, C.; Grøtli, M. Org Biomol Chem 2009, 7, 3421.

[9] Naito, H.; Sugimori, M.; Mitsui, I.; Nakamura, Y.; Iwahana, M.; Ishii, H.; Hirotani, K.; Kumazawa, E.; Ejima, A. Chem Pharm Bull 1999, 47, 1679.

[10] Abu-Hashem, A. A.; El-Shehry, M. F.; Badria, F. A. Acta Pharm 2010, 60, 311.

[11] Porba, K.; Wietrzyk, J.; Opolski, A. Acta Pol Pharm Drug Res 2006, 63, 189.

[12] Svetlik, J.; Veizerova, L; Liptaj, T.; Kubista, J. ARKIVOC 2009, 2009, 79.

[13] Wang, S. Q.; Fang, L.; Liu, X. J.; Zhao, K. Chin Chem Lett 2004, 15, 885.

[14] Li, M.; Wang, S. W.; Wen, L. R.; Qi, W. Y.; Yang, H. Z. Chin J Struct Chem 2005, 24, 64.

[15] Xia, Y.; Dong, Z. W.; Zhao, B. X.; Ge, X.; Meng, N.; Shin, D. S.; Miao, J. Y. Bioorg Med Chem 2007, 15, 6893.

[16] Ohsumi, K.; Matsueda, H.; Hatanaka, T.; Hirama, R.; Umemura, T.; Oonuki, A.; Ishida, N.; Kageyama, Y.; Maezono, K.; Kondo, N. Bioorg Med Chem Lett 2003, 13, 2269. Molecules 2012, 17, 10388.

[17] Kett, W. C.; Batley, M.; Redmond, J. W. Carbohydr Res 2000, 329, 169.

[18] Nishimura, N.; Banno, M.; Maki, A.; Nishiyama, Y.; Maeba, I. ICarbohydr Res 1998, 307, 211.

[19] Michalik, D.; Feist, H.; Peseke, K. Carbohydr Res 2001, 333, 197.

[20] Zsoldos-Mády, V; Csámpai, A.; Szabó, R.; Mészáros-Alapi, E.; Pásztor, J.; Hudecz, F.; Sohár, P. Chem Med Chem 2006, 1, 1119.

[21] Zoorob, H.; Elsherbini, M.; Wafaa, S. Am J Org Chem 2012, 2, 63.

[22] Zhang, J.; Chang, C. W. T. J Org Chem 2009, 74, 685.

[23] Popsavin, M.; Torovic, V.; Kolic, G.; Bogdanovic, G.; Spaic, S.; Popsavin, V. Bioorg Med Chem Lett 2003, 13, 3167.

[24] Popsavin, M., Torovic, L.; Spaic, S.; Stankov, S.; Popsavin, V. ChemInform 2000, 31, doi:10.1002/chin.200044231.

[25] Samb, I.; Pellegrini-Moïse, P.; Chapleur, Y. Tetrahedron Lett 2007, 48, 7978.

[26] (a) Yu, S. M.; Kuo, S. C.; Huang, L. J.; Sun, S. S. M.; Huang, T. F.; Teng, C. M. J Pharm Pharmacol 1992, 44, 667; (b) Huang, L. J.; Hour, M. J.; Teng, C. M.; Kuo, S. C. Chem Pharm Bull 1992, 40, 2547.

[27] (a) Catarzi, D.; Cecchi, L.; Colotta, V.; Filacchioni, G.; Martini, C.; Tacchi, P.; Lucacchini, A. J Med Chem 1995, 38, 1330; (b) Colotta, V.; Catarzi, D.; Varano, F.; Melani, F.; Filacchioni, G.; Cecchi, L.; Trincavelli, L.; Martini, C.; Lucacchini, A. Farmaco 1998, 53, 189.

[28] Foloppe, N.; Fisher, L. M.; Howes, R.; Potter, A.; Robertson Alan, G. S.; Surgenor, A. E. Bioorg Med Chem 2006, 14, 4792.

[29] Kulkarni, A. K. D.; Patil, S. A.; Naik, V. H.; Badam, P. S. Med Chem Res 2011, 20, 346.

[30] Hanumanagoud, H.; Basavaraja, K. M. J Chem Pharm Res 2012, 4, 5165.

[31] Taj, T.; Kamble, R. R.; Kattimani, P. P.; Badami, B. V. Med Chem Res 2012, 21, 3709.

[32] Gowda, S. K. R.; Naik, B. H. S.; Kumar, V. B.; Sudhomani, C. N.; Naik, R. T. R.; Krishnamurthy, G. Spectrochim Acta A, 2013, 105, 229.

[33] Kurdekar, G. S.; Puttanagouda, S. M.; Kulkarni, N. V. et al. Med Chem Res 2011, 20, 421.

[34] Raman, N.; Raja, S. J. J Serb Chem Soc 2007, 72, 983.

[35] Junek, H.; Aigner, H. Chem Ber 1973, 106, 914.

[36] (a) Otto, H. H. Arch Pharm 1974, 307, 444; (b) Otto, H. H.; Schmelz, H. Arch Pharm 1979, 312, 478; (c) Abdel-Latif, F. F. Z. Naturforsch B 1990, 45, 1675; (d) Dyachenko, V. D.; Rusanov, E. B. Chem Heterocycl Compd (Engl Transl) 2004, 40, 231.

[37] (a) Sharanin, Yu A.; Sharanina, L. G.; Puzanova, V. V. J Org Chem USSR (EnglTransl) 1983, 2291; (b) Klokol, G. V.; Krivokolysko, S. G.; Dyachenko, V. D.; Litvinov, V. P. Chem Heterocycl Compd (EnglTransl) 1999, 35, 1183; (c) Shestopalov, A. M.; Yakubov, A. P.; Tsyganov, D. V.; Emel'yanova, Yu M.; Nesterov, V. N. Chem Heterocycl Compd (Engl Transl) 2002, 38, 1180; (d) Dyachenko, V. D.; Chernega, A. N. Russ J Gen Chem (Engl Transl) 2005, 75, 952.

[38] Mamaghani, M.; Nia, R. H.; Shirini, F.; Tabatabaeian, K.; Rassa. M. Med Chem Res 2015, 24, 1916.

[39] Yusuf, M.; Jain, P. Arab J Chem 2012, 5, 93.

[40] Yusuf, M.; Jain, P. Asian J Res Chem 2011, 4, 1103.

[41] Yusuf, M.; Solanki, I. J Saudi Chem Soc 2015, doi: 10.1016/j. jscs.2015.02.002.

[42] Yusuf, M.; Kaur, M.; Jain, P.; Solanki, I. Spectrochim Acta A Mol Biomol Spectrosc 2012, 97, 470.

[43] Srivalli, T.; Satish, K.; Suthakaran, R. Int J Innovative Pharm Res 2011, 2, 172–174.

[44] Mariappan, G.; Sahan, B. P.; Sutharson, L. Indian J Chem 2010, 49B, 1671.

[45] Sharma, A.; Kumar, V.; Khare, R.; Gupta, G.K.; Beniwal, V. Med Chem Res 2014, 24, 1830.