Analysis of compound 109 using 13c nmr spectra

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The ^{13} C NMR spectrum ofcompound 109-114is represented inPlate 40-45. Structural elucidation of109 by ^{13} C NMR spectrum has been described and it was confirmed from the two dimensional NMR reports of109. Assignments for the other compounds109-114made by comparing with compound109. Table 33 lists the chemical shift values of109. A collection of signals resonated in the aliphatic region at 20. 85, 26. 60 and 27. 98 ppm of the three signals in the aliphatic region, signals at 20. 85, 26. 60 and 27. 98 ppm are assigned to C-7, C-6 and C-8, respectively. The C-5 signal is merged with solvent signal. In addition to this, the benzylic carbon signals C-2 and C-4 were resonated at 64. 53 and 62. 52 ppm respectively, whereas the bridgehead carbon C-1 was appeared at 45. 69 ppm. Moreover, a collection of signals appeared in the region 102. 69-131. 40 ppm, which are unambiguously assigned to aryl carbon. A part from the assigned signals, two signals resonated in the downfield at 142. 56 and 142. 72 ppm is assigned to ipso carbons. Another four unassigned signals resonated in the down field region at 159. 55 to 166. 27 ppm and these signals belong to C= 0, C= N, C-OH carbons respectively.

^{1} H- ^{13} C COSY spectra

Plate 46 and 47 represents the ^{1} H- ^{13} C COSY spectrum of105and the correlations showed inTable34. HMBC (Plate 40)and HSQC (Plate 41)correlations have been used to assign the benzylic carbons, ipso carbons of aryl group, and methylene carbons (C-6, C-7, and C-8). The benzylic protons observed at 4. 30 (H-2a) and 4. 25 ppm (H-4a) showed cross peak with 64. 33 (C-2) and 62. 52 ppm (C-4), which may be due to the C-2 and C-4 carbons of the piperidone heterocyclic of the ABN system. The bridgehead methine proton signals at 2. 50 (H-1e) and 2. 98 (H-5e) ppm was correlated

with the carbon signals at 45. 69 ppm (C-1) and 39. 57 ppm (C-5). This indicates that the signals appeared at 45. 69 and 39. 57 ppm is unambigously assigned to C-1 and C-5 carbons. Cross peak with the protons resonated at 2. 77 (H-7a) and 1. 27 (H-7e) ppm was correlated with the carbon signal at 20. 85 ppm and this confirms the signal at 20. 85 ppm was solely specific to C-7 carbon.

H-6a and H-6e protons appeared at 1. 45 and 1. 60 ppm showed cross peak with 26. 60 ppm (C-6 carbon), which confirms that the signal at 26. 60 ppm was due to C-6 carbon and the carbon signal at 20. 85 ppm was attributed to the corresponding C-7 carbon. Similarly, the H-8e (1. 63 ppm) and H-8a (1. 55 pm) protons was correlated with the carbon signal at 27. 98 ppm (C-8), which suppors that the signal at 27. 98 ppm was ascribed to the cyclohexane ring carbon C-8.

Analysis of spectra of 109-114

^{1} H and ^{13} C NMR spectral analysis of other compounds such as N’-(2, 4-bis(4-chlorophenyl)-3-azabicyclo[3. 3. 1]nonan-9-ylidene)-2, 4-dihydro-xybenzohydrazide101, N’-(2, 4-bis(4-fluorophenyl)-3-azabi-cyclo-[3. 3. 1]nonan-9-ylidene)-2, 4-dihydroxybenzohydrazide102, N’-(2, 4-bi-s(4-bromophenyl)-3-azabicyclo[3. 3. 1]nonan-9-ylidene)-2, 4-dihydroxy-benzohydrazide103, N’-(2, 4-dip-tolyl-3-azabicyclo[3. 3. 1] nonan-9-ylidene)-2, 4-dihydroxybenzohydrazide104, N’-(2, 4-bis(2-chloro-phenyl)-3-azabicyclo[3. 3. 1]nonan-9-ylidene)-2, 4-dihydroxy-benzohy-drazide105was analyzed in a similar way of109. The chemical shift and splitting patterns observed using ^{1} H and ^{13} C NMR assignments for other compounds is

presented inTables 35and36and their corresponding spectra are given inPlates 33-37 & 41-45, respectively. Tables 37-42 show the brief description ofanalytical and spectral data ofcompounds 109-114. Taken together, all the above observations substantiate the proposed structure and twin-chair (CC) conformation of 2r, 4c-diaryl-3-azabicyclo [3. 3. 1] nonan-9-one-4-methyl-1, 2, 3-thiadazole-5-carbonyl hydrazones102-108.

Biological study

Free radical scavenging activity

Intensity of the free radical scavenging potential strongly depends upon its chemical structure. Several studies have demonstrated that the free radical scavenging effects is noticeably influenced by the number and position of hydroxyl groups on the D ring and by the extent of conjugation between the D ring amide carbonyl group [1-4]. The dihydroxy structure in the D ring confers high stability to the hydrazones phenoxyl radical *via* hydrogen bonding or by electron delocalization. The amide carbonyl group double bond (Conjugation with the amide oxo group) determines the coplanarity of the phenyl ring and participates in radical stabilization *via* electron delocalization over all ring system [4]. Initial studies on analysis of free radical scavenging potential of diaryl 3-azabicyclononanones revealed a concentration dependent weak anti-radical activity resulting from reduction of DPPH•, ABTS•+, O•−, OH•, and nitric oxide radicals to their non-radical forms. In order to bring the dihydroxy structure in the D ring and the amide carbonyl group double bond (Conjugation with the amide oxo group) into diaryl 3-azabicyclononanones to enhance the free radical potential(figure 14), We

therefore synthesized N’-(2, 4-diaryl-3-azabicyclo[3. 3. 1]nonan-9-ylidene)-2, 4-dihydroxybenzohydrazide(109-114)by the reaction of95, 96, 98-101with 2, 4-dihydroxybenzoic acid hydrazide(94)in the presence of acetic acid.

All the tested compounds showed a concentration dependent anti-radical activity against various free radicals. IC_{50} values for the free radical scavenging effects of ascorbic acid and various synthetic compounds (109-114) are shown in Table 43. This may be due to the hydrogen atom donation mechanism and the electron donation mechanism. In the hydrogen atom transfer mechanism, hydroxyl groups donate hydrogen to a radical stabilizing it and giving rise to a relatively stable hydrazones phenoxyl radical. Figure 15illustrates the probable mechanism of hydrogen atom donating ability of compounds 109-114.

The electron donation mechanism involves through the strong hydrogen bond of -OH moiety with the oxygen atom of amide carbonyl group that may prevent efficient deprotonation and enhance their radical scavenging action by means of hydrogen atom donation. Figure 16illustrates the probable mechanism of free radical scavenging effects of compounds 109-114 through electron donating mechanism. Structure A is the parent neutral molecule of compounds 109-114. Structure B is the initial radical ions and structure C is its more stable tautomeric form. The tautomeric form C of the radical ions results from the initial radical ions B and proton transfer from C-2Êº -OH to carbonyl groups.

We found required structural features to scavenge free radical in our tested compounds (109-144). However, we have noticed a diverse range of effects

against various free radicals. This may be due the different substitution at the C-2 and C-6 positions of the azabicyclononan-9-one moiety. Compound 8 devoid of any substituents at the para position of the phenyl groups at the C-2 and C-6 positions of the azabicyclononan-9-one moiety and phenyl rings with electron-donor methyl groups at the para position of compounds 113 showed excellent free radical scavenging effects compared to standard antioxidant ascorbic acid, a known antioxidant used as a positive control. This may be due to incorporation of methyl groups at para position phenyl ring. Several studies have demonstrated that organic molecules incorporating a methyl groups can act as free radical trapping agents and are capable of opposing oxidative challenges [5, 6]. Compounds possessing electron-withdrawing chloro (110/114), bromo (111), and fluoro (112), substitutions at the para position of the piperidine moiety showed admirable *in vitro* free radical scavenging effects against various free radicals. This admirable or less free radical scavenging effects of compounds with bromo, choloro and fluoro substitutions may be due to the electron-withdrawing inductive effect of halogens. The results obtained in the present study are in line with other findings [7, 8]. Taken together, the current research suggests that azabicyclononane ring ensuring hydroxyl groups on the D ring and by the extent of conjugation between the D ring amide carbonyl group with strong free scavenging effects (111) may conceivably contribute to its protective effects against free radical-induced oxidative stress and carcinogenesis.

Antibacterial and antifungal activity

Synthesized compounds109-114were examined for their antibacterial and antifungal potencies. *In vitro* studies by twofold serial dilution method was adopted. Streptomycin/ streptomycin/ fluconazole were used as a positive control. Table 44shows the MICs of test compounds109-114. Analysis of *in vitro* antimicrobial effects of all the N'-(2r, 4c-diaryl-3-azabicyclo[3. 3. 1]nonan-9-ylidene)-2, 4-dihydroxybenzohydrazide109-114revealed a diverse range of (1. 56-200 µg/mL) against the various bacterias and fungus. The compounds deprived of any substituents at the aryl rings in109hinder the growth of all bacteria and fungus at a MIC value of 100-200 µg/mL. However, compounds110, 111and112possessing *para* halo (electron withdrawing substitutents chloro, fluoro and bromo) substituted aryl groups in azabicyclononane moiety accounts for the enhanced inhibitory effects against *B. subtilis* , *K. pneumonia, P. aeruginosa, S. aureus, A. flavus, A. Niger, C. albicans, and Candida6* at MIC values of 1. 56-25 µg/mL when compared to the standard antibiotic streptomycin/ fluconazole.

Several studies have also documented that electron-withdrawing groups (fluoro, bromo and chloro) substituted azabicyclononan-9-one derivatives exhibited outstanding antibacterial and antifungal activities [9, 10]. Compound114with ortho chloro substituent in the phenyl moiety displays good antibacterial activity against all pathogens. Other compounds displayed reduced inhibitory effects against various bacterial strains compared to the standard streptomycin/ fluconazole. The results of the present study demonstrates that electron withdrawing groups at the para position of the aromatic ring in azabicyclononan-9-one moiety exert superior inhibitory effect against various tested microbes compared to the other test

compounds and standard drug. The SARs based on IC_{50} values (table 44) showed that variations in substitution of the aryl groups at C-2 and C-4 position of the azabicyclononane ring may have significant impact on the anti-microbial activity against various microbes.

Conclusion

The chemical condensation of diversely substituted diaryl 3-azabicyclononan-9-ones with 2, 4-dihydroxybenzoic acid hydrazide in the presence of acetic acid provide corresponding hydrazones 109-114 with increased antioxidant potential and anti-microbial effects. Although various hydrazones exerted the free radical scavenging effets in a good dose-dependent manner, compound 109 and 113 were more active in scavenging free radicals than their parent hydrazones and ascorbic acid. In addition, the results of the antimicrobial activities of hydrazones revealed that compounds 110, 111 and 112 possessing *para* halo (electron withdrawing substitutents chloro, fluoro and bromo) display promising activities against all tested microorganisms. The results of the present study provide a further insight into the structural requirements to develop potential new antioxidants and anti-microbial agents.