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Stereochemical Investigation in the 1,3-Dipolar Cycloadditions of 3-Nitro-2-phenyl-2H-1-benzopyrans to Diazoalkanes: Synthesis and Antimicrobial Activity of Novel Benzopyranopyrazole Derivatives

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Abstract: *A series of 3-nitro-2-phenyl-2H-1-benzopyrans (**3a-h**) were prepared and treated with diazomethane and diazoethane to give various benzopyranopyrazole derivatives namely [1,9-b]dihydro-3a-nitro-4-phenyl[1]benzopyrano[3,4-c]pyrazolines(**4a-h**), 4-phenyl[1]benzopyrano [3,4-c]pyrazoles (**5a-h**) and [1,9-b]dihydro-1-methyl-3a-nitro-4-phenyl[1]benzopyrano[3,4-c]pyrazolines(**6a-h**) respectively. The regio and stereochemical outcome of these cycloadditions are discussed. The benzopyranopyrazole derivatives **4a-h** and **6a-h** were tested for their antimicrobial activities against S. aureus, S. lutea, B. subtilis, E. coli, S. typhosa, S. cerevesciae and C. albicans. Compounds **4a-h** were found to be moderately active against the gram positive bacteria and the fungi that were tested.*

During the last decade, many heterocycles such as nitropyrazoles, nitroimidazoles and nitrooxazoles have been investigated as newer synthetic drugs[1]. Amongst the many classes of such heterocyclics, much attention has been focussed on benzopyranopyrazole class of compounds. The structural similarities of benzopyrano pyrazoles with cannabinoids opened new avenues for their use as narcotic agents and these compounds were patented as CNS depressants[2]. Many biological and industrial applications of these compounds have been well documented. The benzopyranpyrazoles and other pyrazole containing heterocycles are known to be skeletal muscle relaxants[3], bactericides[4], antifertility agents[5], antiallergic[6], antiinflammatory, antiarrhythmic, analgesic, antipyretic, antihypertensive, antiplatelet agents[7], anticancer agents[8] and benzodiazepine receptor ligands[9]. Industrially, these compounds have been used as heat sensitive and pressure sensitive imaging materials in photography[10].

Benzopyranopyrazoles have so far been prepared by intramolecular cycloaddition reactions and via multistep condensation reactions[2,11]. Intermolecular cycloadditions of diazoalkanes leading to the above mentioned class of compounds are yet to be studied in detail[12]. Utility of nitroolefins as dipolarophiles in these cycloadditions is of much interest since these reactions lead to the formation of otherwise difficultly accessible nitropyrazoline adducts, which form excellent intermediates for the preparation of nitropyrazoles and

nitrocyclopropanes[13]. Parham and co-workers[13,14] conducted an exhaustive investigation of diazoalkane cycloadditions to nitroolefins. Dean and co-workers[15] studied the effect of nitro group in the 3- position of 3-nitrochromone and 3-nitrocoumarin upon the outcome of their reaction with diazoalkanes. 3-Nitrocoumarin reacted with diazomethane to give mainly the nitrocyclopropane derivative formed from the unstable benzopyranopyrazoline adduct . Junek and co-workers[16] reported in a similar reaction the formation of the alkylated product, 4-methyl-3-nitrocoumarin. Diazoethane in its reaction with 3-nitrocoumarin induced a more complex situation forming many products. The simple alkylation product, 4-ethyl-3-nitrocoumarin was formed in low yields and the reaction mainly led to the formation of ring expansion and ring contraction products.

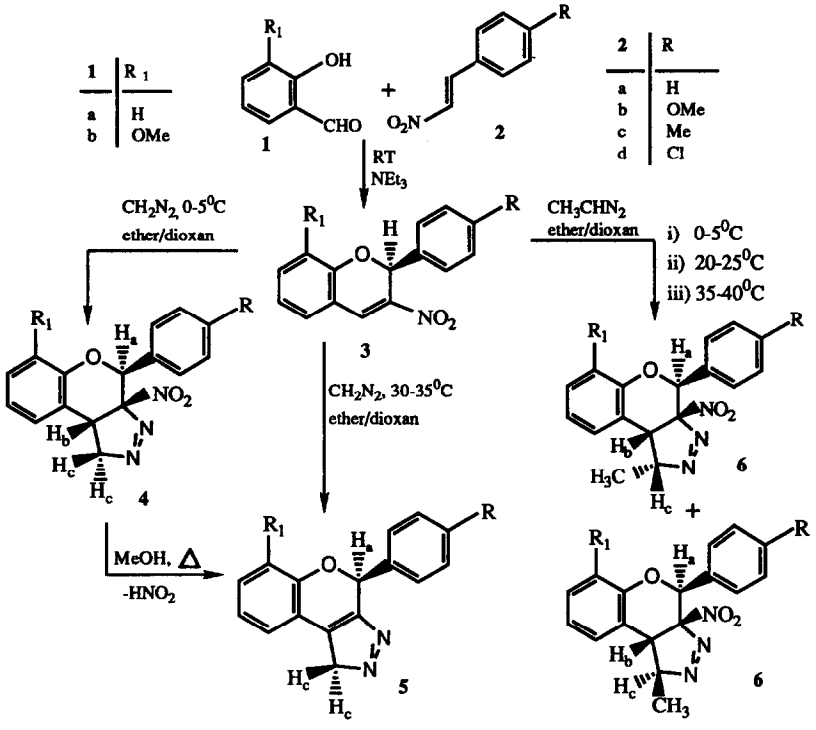
Amongst the several classes of nitroolefins, 3-nitro-2-phenyl-2H-1-benzopyrans are the least explored. Reactions of these compounds with diazoalkanes leading to the formation of polynuclear heterocycles appear to be a virgin field. In continuation of our work on 3-nitro-2-phenyl-2H-1-benzopyrans[17](**3a-h**) and in our efforts to develop benzopyranopyrazoles as novel antimicrobial and antiplatelet agents, these compounds were reacted with diazomethane and diazoethane. Reported abnormalities in the reaction of diazoalkanes with related benzopyrano compounds[15] prompted us to study the above reaction under different conditions. The present investigation has three objectives, i) to achieve a single step synthesis of benzopyrano pyrazoles, ii) to investigate the stereochemical behavior of 2-phenyl-3-nitrochromene derivatives (**3a-h**) towards diazoalkanes in view of the results reported[13-16] in the reactions of 3-nitrocoumarins and 3-nitrochromones with diazoalkanes and iii) to evaluate the antimicrobial activities of the benzopyranopyrazoles thus prepared.

A variety of 3-nitro-2-phenyl-2H-1-benzopyrans (**3a-h**) were synthesized according to the procedure of Sakakibara *et al*[18]. The physical and spectral data were in agreement with those reported in the literature[17]. The diazoalkanes used for the present study were generated freshly from the corresponding nitrosoalkyl ureas. These diazoalkanes were reacted with compounds **3a-h** in two different series of experiments (Scheme 1) .

In the first series of experiments, diazomethane was allowed to react with 3-nitro-2-phenyl-2H-1-benzopyrans. In a typical reaction, equimolar amounts of diazomethane and 3-nitro-2-phenyl-2H-1- benzopyran (**3a**) were reacted at 0-5°C in an ether-dioxane medium. The only product formed quantitatively was recrystallized from pet ether-ethyl acetate to yield a colorless crystalline solid. The compound, m.p 142-43 °C, analyzed for $C_{16}H_{13}N_3O_3$ and was identified as [1,9-b]-dihydro-3a-nitro-4-phenyl-[1]-benzopyrano-[3, 4c]-pyrazoline(**4a**). Friedman and co-workers[19] reported that the end products of the reaction of diazoalkanes with nitroolefins were influenced by the temperature conditions. Hence, the reaction of diazomethane with **3a-h** was studied at a higher temperature range to investigate the outcome. Diazomethane was reacted with 3-nitro-2-phenyl-2H-1-benzopyran (**3a**) at 30-35 °C in dioxane-ether medium. The only product formed, on recrystallization from hexane-ethyl acetate, yielded a colorless crystalline solid, m.p. 207-08 °C and analyzed for $C_{16}H_{12}N_2O$. The analytical and spectral data confirmed that the compound was 4-phenyl[1]benzopyrano[3,4c]-pyrazole (**5a**). The formation of the above product can be rationalized by the thermal loss of HNO_2 from the initially formed nitropyrazoline(**4a**). To confirm the results, [1,9-b]dihydro-3a-nitro-4-phenyl[1]benzopyrano [3,4-c]pyrazoline (**4a**) was warmed in methanol and the resulting product was characterized as 4-phenyl[1]benzopyrano[3,4-c]pyrazole (**5a**) indicating the loss of HNO_2. Apart from pyrazole derivative formed through the elimination of HNO_2 no other rearranged product was noticed. The physical and spectral data of the compounds thus prepared (**4a-h** and **5a-h**) are presented in the experimental section.

Having studied the reaction of diazomethane under two different thermal conditions, the reaction of diazoethane towards 3-nitro-2-phenyl-2H-1-benzopyrans was investigated. These reactions were carried out at three different temperatures (Scheme 1). In a typical reaction, equimolar amounts of diazoethane and 8-methoxy-3-nitro-2-phenyl-2H-1-benzopyran (3e) were reacted at 0-5 ° C in ether-dioxane medium. The resulting product obtained, found to be homogeneous by TLC, was repeatedly recrystallized from hexane-ethyl acetate to yield a colorless crystalline solid.

Scheme 1



3-6	R_1	R	3-6	R_1	R
a	H	H	e	OMe	H
b	H	OMe	f	OMe	OMe
c	H	Me	g	OMe	Me
d	H	Cl	h	OMe	Cl

However, the IR spectrum of the product showed sharp bands at 1555, 1545, 1345 and 1340 cm^{-1} indicating the presence of two nitro groups. The nonhomogeneous nature of the above product was further confirmed from the two sharp bands at 1380 and 1375 cm^{-1} indicating the vibrational modes of the two methyl groups. The ^1H NMR spectrum of the product indicated the presence of two diastereomers. The signals appearing in the spectrum were assigned as follows (ppm): 1.34 (3H, d, J=8 Hz) and 1.96 (3H, d, J=7 Hz) due to the C_1

methyl groups of the two diastereomers, 3.25 (1H, d, J=8 Hz) and 4.00 (1H, d, J=8 Hz) attributed to the H_b proton attached to the 9b carbon. Signals at 3.82 (3H, s) and 3.87 (3H, s) were assigned to the methoxy groups at C_6 carbon. The singlets at 4.75 and 5.40 were attributed to the H_a proton of the two diastereomers. The multiplets appearing in the region 4.52-4.82 and 5.38-5.78 were assigned to the H_c proton attached to C_1 carbon. The complex multiplet in the region 6.62-7.60 represented the aromatic protons. Based on the analytical and spectral data, the product was identified as a diastereomeric mixture of [1,9-b]-benzopyrano[3, 4-c]pyrazoline (**6e**).

Diazoethane is known to add to substituted olefins giving rise to diastereomeric adducts[18]. The percentage composition of the two diastereomers in the above case was determined, based on the integrated area of the signals of H_a proton. In the NMR spectrum of the diastereomeric mixture of **6e**, the H_a signal of the major component appeared at 4.75 and that of the minor isomer at 5.40. The percentage composition of the two diastereomers as determined from the integrated area of H_a signals was found to be 53:47. In most of the cases, one diastereomer was formed in preference over the other. Separation of the diastereomeric mixture into individual components was not possible even after repeated recrystallizations and column chromatography. However, in the case of [1,9-b]dihydro-6-methoxy-1-methyl-3a-nitro-4-phenyl[1]benzopyrano[3,4-c]-pyrazoline diastereomeric mixture (**6e**), the major component could be obtained in pure form after repeated fractional crystallization.

The reaction of diazoethane with 3-nitro-2-phenyl-2H-1-benzopyrans was repeated under two other temperature conditions: 20-25 °C and 35-40 °C. The reaction in both the cases led to the formation of diastereomeric mixtures of the nitropyrazoline adducts **6a-h**. No loss of HNO_2 was observed as was the case with diazomethane and the nitropyrazoline adducts **6a-h** were found to be stable even up to 45 °C. The diastereomeric composition of the reaction product was found to vary with the nature of the substituent in the phenyl ring of the benzopyrano moiety. This may be attributed to the stereoelectronic influence of the methoxy substituent present in the phenyl ring of the benzopyran. The substituents in the phenyl side chain had no effect on the percentage composition of the diastereomeric mixture but were found to play a role in the overall yield of the reaction. The analytical and spectral data of compounds (**6a-h**) are presented in the experimental section.

Stereochemistry of the Cycloadducts Formed in the Reaction of Diazoalkanes with 3-Nitro-2-phenyl-2H-1-benzopyrans

The 3-nitro-2-phenyl-2H-1-benzopyran molecule acquires the conformation in which the 2-phenyl group is quasi-axial due to the dipole-dipole as well as the steric hindrance it experiences with the 3-nitro group (Fig 1a). In its reaction, the diazoalkane approaches the 3-nitro-2-phenyl-2H-1-benzopyran molecule from the side opposite to the C_2-phenyl group due to steric reasons. Hence, in the resulting cycloadduct both 3a-nitro group as well as C_4-phenyl group adopt *cis* disposition with respect to each other (Fig 1b and 1c). Diazomethane reacts with 3-nitro-2-phenyl-2H-1-benzopyran (**3a**) to give [1,9-b]dihydro-3a-nitro-4-phenyl[1]benzopyrano-[3,4-c]-pyrazoline (**4a**) as the sole adduct. The ^1H NMR spectrum of the compound (**4a**) shows the two H_c protons appearing as doublet of doublets at 4.74 and 5.64 ppm respectively. As seen from the molecular model (Fig 1b), the H_c proton which is ß-oriented is in plane with the benzene ring of the benzopyrano moiety as well as with the N=N double bond of the pyrazoline ring and hence being highly deshielded appears at a relatively downfield position (5.64 ppm), whereas the other H_c proton which is in α–orientation is not experiencing the

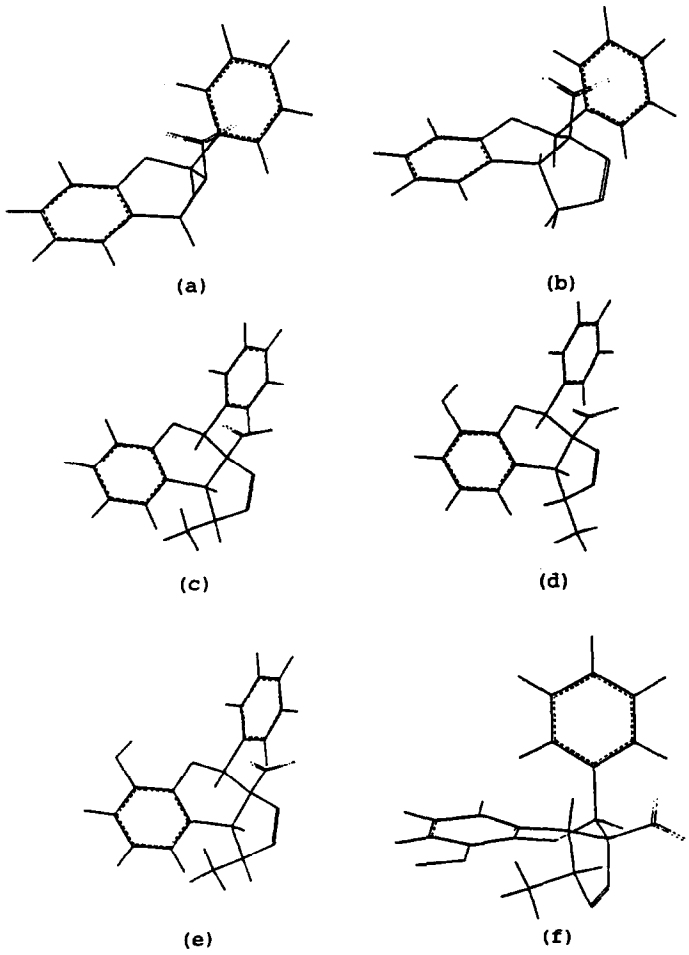


Fig. 1. Molecular models of: a) 3-nitro-2-phenyl-2H-1-benzopyran (**3a**), 'quasi-axial' phenyl group; b) [1,9-b]dihydro-3a-nitro-4-phenyl [1]benzopyrano[3,4-c]pyrazoline(**4a**); c) [1,9-b]dihydro-1-methyl-3a-nitro-4-phenyl[1]benzopyrano[3,4-c]pyrazoline(**6a**), steric interaction between C_1-α–CH_3 group and aromatic C_9-H atom; d) ß-C_1 methyl diastereomer of **6e**; e) α-C_1 methyl diastereomer of **6e**; f) the twist half chair conformation of the α–C_1 methyl diastereomer of **6e** indicating the endo methyl group.

same deshielding effect of the benzene ring and N=N double bond to an extent felt by the ß-H_c atom and hence appears at 4.74 ppm. Diazoethane reacted with 3-nitro-2-phenyl-2H-1-benzopyrans yielding diastereomeric mixtures of the cycloadducts. The reaction of diazoethane with 8-methoxy-3-nitro-2-phenyl-2H-1-benzopyran (**3e**) yielded nearly equal amounts of the diastereomeric mixture of [1, 9-b]dihydro-6-methoxy-1-methyl-3a-nitro-4-phenyl-[1]benzopyrano[3,4-c]pyrazoline (**6e**). Only one isomer could be separated in relatively pure form. Based on the NMR data of the diastereomeric mixture, the following conclusion was drawn.

The C_1-methyl group of the major isomer assumes ß-orientation (as can be seen from the anisotropic influence of 3a-nitro group (Fig 1d) and hence is deshielded to a greater extent (1.96 ppm) as compared to that of α-methyl group of the minor isomer (Fig 1e), which is free from the influence of the nitro group and appears at its usual position (1.34 ppm). Based on the above observation, the major isomer is designated as the ß-isomer and the minor one as the α-isomer, the terms α and ß indicating the orientations of the C_1-methyl groups. The steric interaction of the C_1-methyl group with the aromatic C_9-hydrogen governs the configuration and conformation of the entire molecule. When the C_1-methyl group is in α-orientation as in the minor isomer, the steric interaction between α-C_1-methyl and the aromatic C_9-H is so severe (Fig 1e) that the entire molecule is subjected to conformational change resulting in a totally different geometry (Fig 1f) in which the C_1-methyl group is now in endo position.

The H_b proton of the minor α–isomer experiences a greater anisotropic effect of the nitro group as compared to the H_b proton of the major ß-isomer, thereby appearing at a relatively downfield position of 4.0 ppm vs. 3.25 ppm for the ß-isomer. The 6-methoxy groups of both α and ß-isomers are independent of any preferential shielding or deshielding effects from the molecule and therefore appear close to each other in their normal position around 3.84 ppm. The H_a proton of the major isomer experiences a pronounced shielding effect due to the N=N double bond of the pyrazoline ring (Fig 1d) and appears upfield at 4.75 ppm as compared to the H_a proton of the α-isomer which appears at 5.4 ppm (Fig 1f). The Hc proton of the minor α-isomer (Fig 1f) being in the plane of the benzene ring of the benzopyrano moiety experiences a high deshielding effect and appears at a downfield position of 5.38-5.78 ppm as compared to the H_c proton of the ß-isomer (Fig 1d) appearing at 4.52-4.82 ppm. The predominance of the ß-C_1-methyl isomer over the α-C_1-methyl may be rationalized by the decreased steric strain that the transition state of the ß-methyl oriented isomer experiences between the methyl group of diazoethane and the aromatic protons of the benzopyrano moiety of 3-nitro-2-phenyl-2H-1-benzopyrans as compared to the transition state of the α-methyl oriented isomer.

ANTIMICROBIAL ACTIVITY

The biological activity of synthetic and naturally occurring nitro compounds was reviewed by Eckstein *et al*[20]. Several nitro compounds were known to be therapeutically important in the treatment of infectious diseases. In view of the significant biological activity of nitro compounds, benzopyranopyrazoles and pyrazolines, we report herein the antimicrobial activity of compounds **4a-h** and **6a-h** against three gram positive, two gram negative bacteria and two species of fungi. The minimum inhibitory concentrations (MIC) of these compounds were evaluated against each of the microorganisms using the broth dilution technique[21] and are reported in Table 1. The compounds obtained from diazomethane cycloadditions **4a-h** were very active against the gram positive bacteria and fungi (MIC: 25-75 µg/mL). The gram negative bacteria were not very susceptible to the same molecules.The compounds resulting from diazoethane cycloadditions, **6a-h** were moderately active against the fungal species (MIC: 100-250 µg/mL), slightly active against S. typhosa (MIC: 250 µg/mL) and inactive against the gram positive bacteria. These compounds were not found to be superior to the antibiotics used as the standards in test systems.

Table 1: Minimum Inhibitory Concentrations (μg/mL) of Compounds 4(a-h) and 6(a-h).

Compound	Gram Positive Bacteria			Gram Negative Bacteria		Yeasts	
	Sa	Bs	Sl	Ec	St	Sc	C
4a	75	200	25	-	-	-	25
4b	75	50	50	100	100	75	75
4c	250	-	100	-	-	75	50
4d	50	-	-	75	-	-	75
4e	75	100	50	-	-	-	25
4f	50	25	50	-	-	75	75
4g	-	-	50	-	250	75	100
4h	50	-	-	100	-	-	75
6a	-	-	-	-	250	-	-
6b	-	-	-	250	-	100	200
6c	-	-	-	-	-	200	250
6d	-	-	-	-	-	100	200
6e	-	-	-	-	250	-	-
6f	-	-	-	-	-	100	200
6g	-	-	-	-	250	100	100
6h	-	-	-	-	-	100	100
S	5	-	-	-	-	-	-
A	2	1	-	-	-	-	-
N	-	-	-	-	-	-	3

Sa: Streptomyces aureus, Bs: Bacillus subtilis, Sl: Sarcina lutea, Ec: Escherichia coli,
St: Salmonella typhosa, Sc: Saccharomyces cerevesciae, Ca: Candida albicans, S:Streptomycin,
A: Ampicillin, N: Nystatin.

EXPERIMENTAL SECTION

All melting points are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 257-B spectrophotometer using KBr pellets. ^{1}H-NMR spectra were recorded on a Varian XL-100 NMR spectrometer ($CDCl_3$, TMS as internal standard, s:singlet, d:doublet, t:triplet, m:multiplet, dd:doublet of a doublet). Mass spectra were recorded on a Varian MAT-112 mass spectrometer (EI, 70 eV). Energy minimized molecular models (Fig 1) were constructed using SYBYL 6.0 software on IRIS indigo Elan 4000 workstation. Preparation of β-nitrostyrenes(**2**) and 3-nitro-2-phenyl-2H-1-benzopyrans(**3a-h**) was previously reported[17].

General Procedure for the Synthesis of [1,9-b]Dihydro-3a-nitro-4-phenyl[1]benzopyrano [3,4-c]pyrazolines(4a-h). In a typical reaction, 3-nitro-2-phenyl-2H-1-benzopyran (5 mmol) was dissolved in dioxane-ether (10 mL) and maintained at 0°C in an ice bath. To this was added dropwise with stirring freshly prepared diazomethane (5 mmol) in ether (10 mL) over a period of 1 hr. The stirring was continued at 0°C for another 2 hrs. The solid mass formed at the end of the reaction was filtered and purified by recrystallization from hexane-ethyl acetate. The mother liquor remaining in the reaction flask was concentrated and subjected to column chromatography to yield additional amount of the above product.

[1,9-b]Dihydro-3a-nitro-4-phenyl[1]benzopyrano[3,4-c]pyrazoline (4a): Yield: 90%. M.P. 142-143 °C. (Found: C,65.20; H,4.57; N,14.36. $C_{16}H_{13}N_3O_3$ requires C,65.08; H,4.41; N,14.24). IR(ν_{max}, cm^{-1}) 1550,1355. ^1H NMR 3.86 (1H,dd,H_b,$J_{b,c}$=9Hz), 4.74 (1H, dd,H_c,$J_{c,c}$=18Hz), 5.08 (1H,s,H_a), 5.62 (1H,dd,H_c,$J_{c,c}$=18Hz), 6.96-7.30 (4H,m,Ar-H), 7.48-7.56 (5H,m,Ar-H). m/z 295(M^+).

[1,9-b]Dihydro-4-(4'-methoxy)phenyl-3a-nitro[1]benzopyrano[3,4-c]pyrazoline (4b): Yield: 82% M.P. 147-48 °C. (Found: C,62.88; H,4.60; N,12.89. $C_{17}H_{15}N_3O_4$ requires C,62.77; H4.62; N,12.92). IR(ν_{max},cm^{-1}) 1548,1352. ^1H NMR 3.87 (3H,s,OCH_3), 3.76-3.98 (1H,m,H_b), 4.75 (1H,dd,H_c,$J_{c,c}$=18Hz), 5.12 (1H,s,H_a), 5.60 (1H,dd,H_c,$J_{c,c}$=18Hz), 6.80-7.46 (9H,m,Ar-H). m/z 325(M^+).

[1,9-b]Dihydro-4-(4'-methyl)phenyl-3a-nitro[1]benzopyrano[3,4-c]pyrazoline (4c): Yield: 86%. M.P. 139-40 °C. (Found: C,66.20; H, 4.94 ; N,13.68. $C_{17}H_{15}N_3O_3$ requires C,66.02; H,4.85; N, 13.59). IR(ν_{max},cm^{-1}) 1551,1340. ^1H NMR 2.41 (3H,s,CH_3), 3.87 (1H,dd,H_b,$J_{b,c}$=9Hz), 4.74 (1H,dd,H_c,$J_{c,c}$=18Hz), 5.09 (1H,s,Ha), 5.62 (1H,dd,H_c,$J_{c,c}$=18Hz), 6.92-7.44 (8H,m,Ar-H). m/z 309(M^+).

[1,9-b]Dihydro-4-(4'-chloro)phenyl-3a-nitro[1]benzopyrano[3,4-c]pyrazloine (4d): Yield: 74%. M.P. 138-39 °C. (Found: C,58.36; H, 3.86; N,12.87. $C_{16}H_{12}N_3O_3Cl$ requires C,58.27; H,3.64; N,12.75). IR(ν_{max},cm^{-1}) 1552,1340. ^1H NMR 3.84 (1H,dd,H_b,$J_{b,c}$=9Hz), 4.74 (1H,dd,H_c,$J_{c,c}$=18Hz), 5.00 (1H,s,H_a), 5.66 (1H,dd,H_c,$J_{c,c}$=18Hz), 6.96-7.22 (4H,m,Ar-H), 7.34-7.56 (4H,m,Ar-H). m/z 331(M^+).

[1,9b]Dihydro-6-methoxy-3a-nitro-4-phenyl[1]benzopyrano[3,4-c]pyrazoline (4e): Yield: 80%. M.P.154-55 °C. (Found: C,62.84; H,4.69 ; N,12.80. $C_{17}H_{15}N_3O_4$ requires C,62.76; H,4.61; N,12.92). IR(ν_{max},cm^{-1}) 1558,1350. ^1H NMR 3.85 (3H,s,OCH_3), 3.82-3.98 (1H,m,H_b), 4.76 (1H,dd,H_c,$J_{c,c}$=18Hz), 5.22 (1H,s,H_a), 5.60 (1H,dd,H_c,$J_{c,c}$=18Hz), 6.72-7.14 (3H,m,Ar-H), 7.34-7.56 (5H,m,Ar-H). m/z 325(M^+).

[1,9b]Dihydro-6-methoxy-4-(4'-methoxy)phenyl-3a-nitro[1]benzopyrano[3,4-c]pyrazoline (4f): Yield: 84%. M.P 161-62 °C. (Found: C,60.83; H,4.67; N,11.87. $C_{18}H_{17}N_3O_5$ requires C,60.85; H,4.79; N,11.83). IR(ν_{max},cm^{-1}) 1552,1345. ^1H NMR 3.80 (3H,s,OCH_3), 3.88 (3H,s,OCH_3), 3.82-3.96 (1H,m,H_b), 4.76 (1H,dd,H_c,$J_{c,c}$=18Hz), 6.92-7.18 (3H,m,Ar-H), 7.26-7.62 (4H,m,Ar-H). m/z 355(M^+).

[1,9-b]Dihydro-6-methoxy-4-(4'-methyl)phenyl-3a-nitro[1]benzopyrano[3,4-c]pyrazoline (4g): Yield: 65%. M.P.152-53 °C. (Found: C,63.87; H,5.19; N,12.48. $C_{18}H_{17}N_3O_4$ requires C,63.72; H,5.01; N, 12.39). IR(ν_{max},cm^{-1}) 1550,1345. ^1H NMR 2.37 (3H,s,CH_3), 3.84 (3H,s,OCH_3), 3.80-4.00 (1H,m,H_b), 4.75 (1H,dd,H_c,$J_{c,c}$=18Hz), 5.26 (1H,sH_a), 5.56 (1H,dd,H_c,$J_{c,c}$=18Hz), 6.68-6.88 (3H,m,Ar-H), 6.92-7.42 (4H,m,Ar-H). m/z 339(M^+).

4-(4'-chloro)phenyl-[1,9-b]Dihydro-6-methoxy-3a-nitro[1]benzopyrano[3,4-c]pyrazoline (4h): Yield: 70%. M.P 158-59 °C. (Found: C,56.62; H,3.98; N,11.76. $C_{17}H_{14}N_3O_4Cl$ requires C,56.75; H,3.89; N,11.68). IR(ν_{max},cm^{-1}) 1548,1340. ^{1}H NMR 3.86 (3H,s,OCH_3), 3.78-3.96 (1H,m,H_b), 4.74 (1H, dd,H_c,$J_{c,c}$=18Hz), 5.16 (1H,s,H_a), 5.54 (1H,dd,H_c,$J_{c,c}$=18Hz), 6.74-7.18 (3H,m,Ar-H), 7.26-7.58 (4H,m,Ar-H). m/z 361(M+).

General Procedure for the Synthesis of 4-Phenyl[1]benzopyrano[3,4-c]pyrazoles(5a-h). In a typical reaction, 3-nitro-2-phenyl-2H-1-benzopyran (5 mmol) was dissolved in dioxane-ether (10 mL) and stirred at room temperature. To this was added dropwise with stirring freshly prepared diazomethane (5 mmol) in ether (10 mL) over a period of 1 hr. The stirring was continued for another two hrs. The solid formed at the end of the reaction was filtered and purified by recrystallization from hexane-ethyl acetate. The reaction mixture yielded additional amounts of the above solid upon concentration and silica gel chromatography.

4-Phenyl[1]benzopyrano[3,4-c]pyrazole (5a): Yield: 62%. M.P. 207-08 °C. (Found: C,77.60; H,4.84; N,11.48. $C_{16}H_{12}N_2O$ requires C,77.42; H,4.84; N,11.29). IR(ν_{max},cm^{-1}) 3100,1600,1580. ^{1}H NMR 6.45 (1H,s,H_a), 6.96-7.60 (9H,m,Ar-H), 7.78 (1H,s,H_c). m/z 309(M+).

4-(4'-Methoxy)phenyl[1]benzopyrano[3,4-c]pyrazole (5b): Yield: 65%. M.P. 195-96 °C. (Found: C,73.30; H,5.16; N,10.00. $C_{17}H_{14}N_2O_2$ requires C,73.38; H,5.04; N,10.07). IR(ν_{max},cm^{-1}) 3120,1615. ^{1}H NMR 3.83 (3H,s,OCH_3), 6.38 (1H,s,H_a), 6.82-7.20 (4H,m,Ar-H), 7.26-7.50 (4H,m,Ar-H), 7.74 (1H,s,H_c). m/z 339(M+).

4-(4'-Methyl)phenyl[1]benzopyrano[3,4-c]pyrazole (5c): Yield: 54%. M.P. 198-99 °C. (Found: C,77.97; H,5.30; N,10.78. $C_{17}H_{14}N_2O$ requires C,77.86; H,5.34; N,10.69). IR(ν_{max},cm^{-1}) 3110,1620,1586. ^{1}H NMR 2.38(3H,s,CH_3), 6.38 (1H,s,H_a), 6.88-7.46 (8H,m,Ar-H), 7.70 (1H,s,H_c). m/z 323(M+).

4-(4'-Chloro)phenyl[1]benzopyrano[3,4-c]pyrazole (5d): Yield: 72%. M.P. 172-73 °C. (Found: C,68.03; H,3.97; N,10.01. $C_{16}H_{11}N_2OCl$ requires C,67.96; H,3.89; N9.91). IR(ν_{max},cm^{-1}) 3130,1615,1585. ^{1}H NMR 6.41 (1H,s,H_a), 6.92-7.24 (4H,m,Ar-H), 7.32-7.50 (4H,m,Ar-H), 7.70 (1H,s,H_c). m/z345(M+).

6-Methoxy-4-phenyl[1]benzopyrano[3,4-c]pyrazole (5e): Yield: 80%. M.P. 168-69 °C. (Found: C,73.46; H,5.17; N,10.15. $C_{17}H_{14}N_2O_2$ requires C,73.38; H,5.04; N,10.07). IR(ν_{max},cm^{-1}) 3120,1614,1580. ^{1}H NMR 3.93 (3H,s,OCH_3), 6.54 (1H,s,H_a), 6.74-6.90 (3H,m,Ar-H), 7.30-7.60,Ar-H), 7.30-7.60 (4H,m,Ar-H), 7.64 (1H,s,H_c). m/z 339(M+).

4-(4'-methoxy)phenyl-6methoxy[1]benzopyrano[3,4-c]pyrazole (5f): Yield: 85%. M.P.183-84 °C. (Found: C,70.26; H,5.28; N,9.15. $C_{17}H_{16}N_2O_3$ requires C,70.13; H,5.19;N,9.09). IR(ν_{max},cm^{-1}) 3140,1618,1580. ^{1}H NMR 3.80 (3H,s,OCH_3), 3.88 (3H,s,OCH_3), 6.45 (1H,s,H_a), 6.72-7.04 (3H,m,Ar-H), 7.36-7.48 (4H,m,Ar-H), 7.52 (1H,s,H_c). m/z 369(M+).

4-(4'-Methyl)phenyl-6-methoxy[1]benzopyrano[3,4-c]pyrazole (5g): Yield: 78%. M.P. 176-77 °C (Found: C,73.79; H, 5.59; N,9.67. $C_{18}H_{16}N_2O_2$ requires C,73.97; H,5.48; N,9.59). IR(ν_{max},cm^{-1}) 3140,1620,1588. ^{1}H NMR 2.33 (3H,s,CH_3), 3.89 (3H,s,OCH_3), 6.47 (1H,s,H_a), 6.70-7.46 (7H,m,Ar-H),

7.55 (1H,s,H_c). m/z 353(M^+).

4-(4'-Chloro)phenyl-6-methoxy[1]benzopyrano[3,4-c]pyrazole (5h): Yield: 70%. M.P. 174-75 °C. (Found: C,65.21; H,4.29; N,8.99. $C_{17}H_{13}N_2O_2Cl$ requires C,65.28; H,4.16; N,8.96). IR(ν_{max},cm^{-1}) 3110,1618,1586. ^1H NMR 3.91 (3H,s,OCH_3), 6.47 (1H,s,H_a), 6.72-7.46 (7H,m,Ar-H), 7.52 (1H,s,H_c). m/z 375(M^+).

General Procedure for the Synthesis of [1,9-b]Dihydro-1-methyl-3a-nitro-4-phenyl[1]-benzopyrano[3,4-c]pyrazolines(6a-h). The reactions were carried out under three different temperature conditions: i) 0-5 ° C, ii) 25-35 ° C and iii) 40-45 ° C. In a typical reaction, 3-nitro-2-phenyl-2H-1-benzopyran (5 mmol) was dissolved in dioxane-ether (10 mL). To this was added freshly prepared diazoethane (5 mmol) in ether-dioxane (10 mL) over a period of 2 hrs. The reaction was allowed to continue for another two hrs. The solid formed at the end of the reaction was filtered and purified by recrystallization from hexane-ethyl acetate.

[1,9-b]Dihydro-1-methyl-3a-nitro-4-phenyl[1]benzopyrano[3,4-c]pyrazoline (6a): Yield: 68%. Diastereomeric mixture (97:3). (Found: C,66.29; H,4.84; N,13.65. $C_{17}H_{15}N_3O_3$ requires C,66.01; H,4.85; N,13.59). IR(ν_{max},cm^{-1}) 1552,1540,1355,1380. ^1H NMR 1.98 (3H,d,CH_3,J=8Hz), 3.21 (1H,d,H_b,J=8Hz), 4.42-4.78(1H,m,H_c), 4.66 (1H,s,H_a), 7.00-7.58 (9H,m,Ar-H). m/z 309(M^+).

[1,9-b]Dihydro-4(4'-methoxy)phenyl-1-methyl-3a-nitro[1]benzopyrano[3,4-c]pyrazoline (6b): Yield: 62%. Diastereomeric mixture (98:2). (Found: C,63.66; H,4.91; N,12.57. $C_{18}H_{17}N_3O_4$ requires C,63.71; H,5.01; N,12.38). IR(ν_{max},cm^{-1}) 1550,1545,1365,1380. ^1H NMR 1.96 (3H,d,CH_3,J=8Hz), 2.40 (3H,s,OCH_3), 3.20 (1H,d,H_b,J=8Hz), 4.64 (1H,s,H_a), 4.42-4.76 (1H,m,H_c), 6.98-7.18(4H,m,Ar-H),7.22-7.46(4H,m,Ar-H). m/z 339(M^+).

[1,9-b]Dihydro-1-methyl-4(4'-methyl)phenyl-3a-nitro[1]benzopyrano[3,4-c]pyrazoline (6c): Yield: 65%. Diastereomeric mixture (88:12). (Found: C,66.79; H,5.22; N,12.83. $C_{18}H_{17}N_3O_3$ requires C,66.87; H,5.26; N,13.00). IR(ν_{max},cm^{-1}) 1555,1548,1365,1380. ^1H NMR 1.33 (3H,d,CH_3,J=8Hz), 1.97 (3H,d,C_1-CH_3,J=7Hz), 2.36 (3H,s,CH_3), 2.41 (3H,s,CH_3), 3.22 (1H,d,H_b,J=8Hz), 3.96 (1H,d,H_b,J=8Hz), 4.65 (1H,s,H_a), 4.45-4.78 (1H,m,H_c), 5.23 (1H,s,H_a), 5.52-5.74 (1H,m,H_c), 7.00-7.48 (16 H,m,Ar-H). m/z 323(M^+).

4-(4'-Chloro)phenyl[1,9-b]dihydro-1-methyl-3a-nitro[1]benzopyrano[3,4-c]pyrazoline (6d): Yield: 70%. Diastereomeric mixture (94:6). (Found: C,59.55; H,4.18; N,12.18. $C_{17}H_{13}N_3O_3Cl$ requires C,59.38; H,4.07; N,12.22). IR(ν_{max},cm^{-1}) 1552,1548,1340,1380. ^1H NMR 1.96 (3H,d,CH_3,J=7Hz), 3.19 (1H,d,H_b,J=8Hz), 4.44-4.78 (1H,m,H_c), 4.60 (1H,s,H_a), 7.02-7.52 (8H,m,Ar-H). m/z 345(M^+).

[1,9-b]Dihydro-6-methoxy-1-methyl-3a-nitro-4-phenyl[1]benzopyrano[3,4-c]pyrazoline (6e): Yield: 64%. Diastereomeric mixture (53:47). (Found: C,63.67; H, 5.16; N,12.26. $C_{18}H_{17}N_3O_4$ requires C,63.71; H,5.01; N,12.38). IR(ν_{max},cm^{-1}) 1555,1545,1340,1375. ^1H NMR 1.34 (3H,d,C_1-CH_3,J=8Hz), 1.96 (3H,d,C_1-CH_3,J=7Hz), 3.25 (1H,d,H_b,J=8Hz), 4.00 (1H,d,H_b,J=8Hz), 3.82 (3H,s,OCH_3), 3.87 (3H,s,OCH_3), 4.52-4.80(1H,m,H_c), 4.75 (1H,s,H_a), 5.54-5.74 (1H,m,H_c), 6.72-7.60 (16H,m,Ar-H).m/z 339(M^+).

[1,9-b]Dihydro-6-methoxy-4-(4'-methoxy)phenyl-1-methyl-3a-nitro[1]benzopyrano[3,4-c]-pyrazoline (6f): Yield: 76%. Diastereomeric mixture (70:30). (Found: C,61.85; H, 5.28; N,11.50.

$C_{19}H_{19}N_3O_5$ requires C,61.78; H,5.14; N,11.38). IR(ν_{max},cm^{-1}) 1550,1548,1350,1385. ^{1}H NMR 1.33 (3H,d,C_1-CH_3,J=8Hz), 1.94 (3H,d,C_1-CH_3,J=7Hz), 3.25 (1H,d,H_b,J=8Hz), 4.01 (1H,d,H_b,J=8Hz), 3.80 (3H,s,OCH_3), 3.83 (3H,s,OCH_3),3.86(3H,s,OCH_3), 3.88(3H,s,OCH_3), 4.48-4.80(1H,m,H_c), 4.74 (1H,s,H_a), 5.40-5.70 (1H,m,H_c), 5.43(1H,s,H_a), 6.74-7.50 (14H,m,Ar-H). m/z 369(M+).

[1,9-b]Dihydro-6-methoxy-1-methyl-4-(4'-methyl)phenyl-3a-nitro[1]benzopyrano[3,4-c]-pyrazoline (6g): Yield: 48%. Diastereomeric mixture (64:36). (Found:C, 64.69; H, 5.21; N,11.99. $C_{20}H_{19}N_3O_4$ requires C,64.58; H,5.38; N,11.89). IR(ν_{max},cm^{-1}) 1550,1548,1350,1385. ^{1}H NMR 1.32 (3H,d,C_1-CH_3,J=7Hz), 1.93 (3H,s,C_1-CH_3,J=7Hz), 2.33 (3H,s,CH_3), 2.37 (3H,s,CH_3), 3.24 (1H,d,H_b,J=8Hz), 4.01 (1H,d,H_b,J=8Hz), 3.79 (3H,s,OCH_3), 3.85 (3H,s,OCH_3), 4.44-4.86 (1H,m,H_c), 4.74(1H,s,H_a), 5.40-5.78(1H,m,H_c), 5.42 (1H,s,H_a), 6.72-7.46(14H,m,Ar-H). m/z 353(M+).

4-(4'-Chloro)phenyl[1,9-b]dihydro-6-methoxy-1-methyl-3a-nitro[1]benzopyrano[3,4-c]-pyrazoline (6h): Yield: 52%. Diastereomeric mixture (65:35). (Found: C,57.96; H,4.21 N,11.39. $C_{17}H_{14}N_3O_4Cl$ requires C,57.83; H,4.28; N,11.24). IR(ν_{max},cm^{-1}) 1550,1546,1340,1378. ^{1}H NMR 1.33 (3H,d,C_1-CH_3,J=7Hz), 1.96 (3H,d,C_1-CH_3,J=7Hz), 3.24 (1H,d,H_b,J=8Hz), 3.98 (1H,d,H_b,J=8Hz), 3.83 (3H,s,OCH_3), 3.88 (3H,s,OCH_3), 4.48-4.82 (1H,m,H_c), 4.68 (1H,s,H_a), 5.55-5.80 (1H,m,H_c), 5.29 (1H,s,H_a), 6.72-7.48 (14H,m,Ar-H). m/z 375(M+).

Experimental Antimicrobial Tests. *S . aureus, B . subtilis, S . lutea, E . coli, S . typhosa, S . cerevesciae and C . albicans* were all obtained as lyophilized preparations from the National Chemical Laboratories, Pune, India. The bacteria were subcultured on nutrient agar and nutrient broth, while the fungi were grown on Sabauraud agar. The MIC of each of the compounds was determined on nutrient agar for bacteria and Sabauraud agar for fungi and these are presented in Table 1. Inocula of the microbial species were prepared by picking colonies of each after overnight growth on a nutrient agar or a Sabauraud agar slant. The cells were resuspended in a sterile nutrient broth to give a concentration of 108 colony forming units (cfu/mL). The inocula were applied to plates containing compounds (1 mg dissolved in 1 mL propylene glycol) in a serial two-fold dilution in the range of 25 µg/mL-250 µg/mL. The plates were incubated at 37 °C for 16-18 hrs for bacteria and at 28 °C for fungi. Ampicillin, streptomycin and nystatin were used as reference standards for the above tests. The MIC was defined as the lowest concentration of the compound that inhibited visible growth of the microorganism to fewer than 10 colonies after 16-18 hrs incubation.

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