Research Paper

Synthesis and Antiinflammatory Activity of Newer Pyrazolinylbenzidines and Isoxazolinylbenzidines

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Sharma and Saxena: Synthesis and Evaluation of Pyrazolinylbenzidines and Isoxazolinylbenzidines

In an effort to search for more active antiinflammatory agent, a series of pyrazolinylbenzidines and isoxazolylbenzidines was designed, synthesized, and screened for their potential as novel orally inflammation inhibitors. Compounds 4,4'-bis-(1''-acetyl-5''-substitutedaryl-2''-pyrazolin-3''-yl)benzidines (8-13) and 4,4'-bis-(2''-substitutedaryl-isoxazolin-4'-yl)benzidines (14-19) have been synthesized from 4,4'-bis-(substituted benzylidenyl-acetyl)benzidines (2-7). The structures of the products have been delineated by spectral and elemental analysis. Compounds 2-19 evaluated for antiinflammatory activity and acute toxicity and results are reported. The compound 4,4'-bis-[1''-acetyl-5''-(*p*-methoxyphenyl)-2''-pyrazolin-3'-yl)benzidine (9) showed more potent and dose-dependent antiinflammatory activity in comparison to reference drug.

Key words: Pyrazolinylbenzidines, isoxazolinylbenzidines, antiinflammatory activity, ulcerogenic liability, toxicity

Inflammation in one form or the other is at the root of most of the common ailments starting from traumatic disorder or fever associated with infection to major life threatening diseases like myocardial infarction or brain hemorrhage or infract. In addition, inflammatory diseases also include various kinds of arthritis. Although many specific drugs like nonsteroidal antiinflammatory drugs (NSAIDs) are widely used with success to combat inflammation with its accompanying pain and fever. However, NSAIDs have high incidence of serious side effects like gastrointestinal ulceration and hypersensitivity. To overcome these limitations search is ongoing throughout the World to find new effective and safe antiinflammatory agent.

Literature survey has revealed that to treat inflammatory disorders, numerous NSAIDs belonging to different chemical groups have been developed but such researches could not win the desired results. Pyrazoline derivatives have been one of such groups, which has been widely explored for the development of new antiinflammatory agents[1-5]. Further, it has also been observed that isoxazoline congeners exhibited promising antiinflammatory activity[6-8]. Bansal *et al.*[9] brought out new compounds of benzidine, which worked as potential antiinflammatory agents. These facts prompted us to synthesize new benzidine derivatives by incorporating pyrazolinyl and isoxazolinyl moieties with a hope to develop more effective antiinflammatory agents.

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MATERIALS AND METHODS

Thermonic melting point apparatus was used in order to determine the melting points of compounds and are not corrected. Thin layer chromatography (TLC) checked the purity of compounds by using silica gel 60 F 254 pre-coated TLC plates (E. Merck). Chromatographic separations were carried out on silica gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck). Elemental analyses (C, H, N) were done on CarloErba-1108 elemental analyzer, and obtained results were found within the ±0.4% of theoretical values. Perkin Elmer-881 and Paragon 500 FTIR infrared spectrophotometer were employed for the determination of functional group in compounds, and v was marked in cm^{-1}. Bruker DRX-300 FT NMR spectrometer was used to get the ^1H NMR spectra at 300 MHz by employing $CDCl_3$/DMSO-d_6 solvent. Chemical shifts are given in δ (ppm) using tetramethylsilane

as internal standard. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Jeol-JMS D-300 instrument was involved in recording the mass spectra. Reaction sequences leading to synthesis of present series is outlined in Scheme 1.

4,4'-Bis-acetyl-benzidine (1):

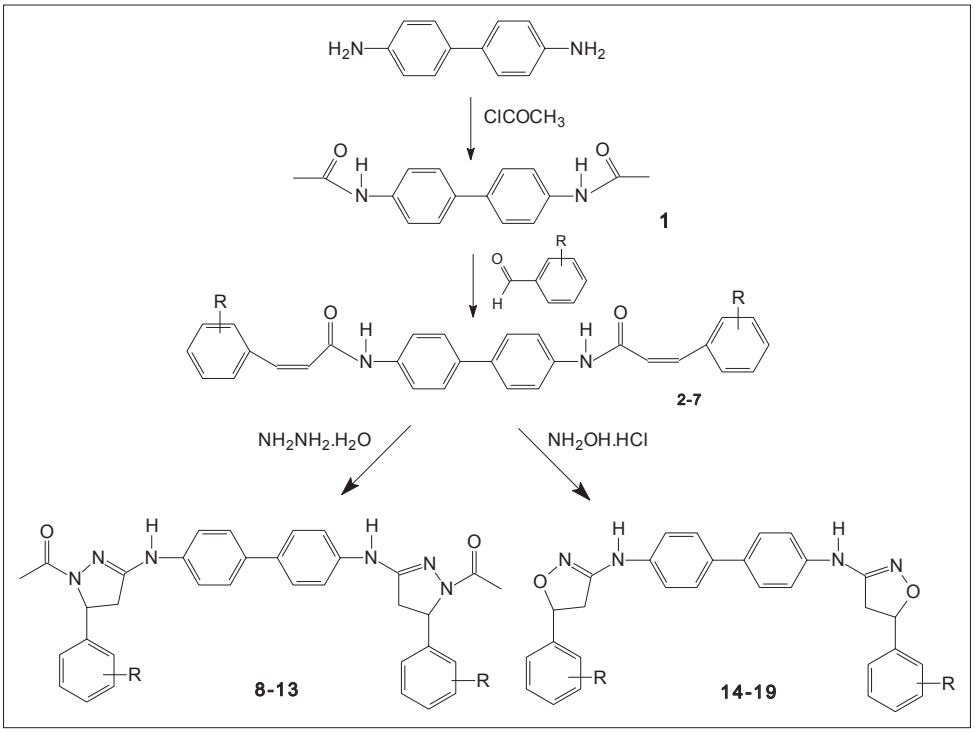
To a solution of benzidine (80.0 g, 0.435 mol) in dry benzene (200 ml), acetyl chloride (61.83 ml, 0.788 mol) was added drop-wise during an hour with constant stirring at 0-5^0. The reaction mixture was further stirred vigorously for 4 h at room temperature, and then refluxed for 6 h on water bath. Excess of solvent was distilled off and the residue thus obtained and drained over the ice. Further, the filtered solid was crystallized from methanol to afford compound 1: (56.0 g, 70%) as colorless crystals; MP (^{0}) 314-315; IR (KBr pellets) ν cm^{-1}: 3277 (N-H), 3015 (C-H, aromatic), 2855 (C-H of $COCH_3$), 1659 (C=O), 1570 (C····C of aromatic ring); ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 8.65 (s, 2H, 2 × NHCO, exchangeable with D_2O), 7.30-7.90 (m, 8H, Ar-H), 2.55 (s, 6H, 2 × $COCH_3$); MS: $[M]^+$ at 268 (m/z); Analysis calculated for $C_{16}H_{16}O_2N_2$: C, 71.64; H, 5.97; N, 10.45. Found: C, 71.40; H, 5.65; N, 10.77.

4,4'-Bis-(benzylidenylacetyl)benzidine (2):

A solution of compound 4,4'-bis-acetylbenzidine 1 (7.0 g, 0.026 mol) in methanol (75 ml) with benzaldehyde (2.66 ml, 0.026 mol) and few drops of 2% NaOH solution was heated under reflux for 11 h, while reaction progress and completion was observed by TLC. The post process was to distill the reaction mixture and then it was cooled down, further the remnant solid passed through the cold water. Taking the process ahead the substance was filtered to obtain the solid mass, which was later on crystallized from acetic acid giving compound 2 (4.34 g, 62%) as colorless crystals; MP (^{0}) 149-150; IR (KBr pellets) ν cm^{-1}: 3280 (N-H), 3022 (C-H, aromatic), 2940 (C-H, aliphatic), 1665 (C=O), 1577 (C····C of aromatic ring), 1218 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 8.82 (s, 2H, 2 × NHCO, exchangeable with D_2O), 8.41 (d, J=7.0 Hz, 2H, 2 × Ar-CH), 7.82-7.06 (m, 18H, Ar-H), 6.52 (d, J =11.0 Hz, 2H, 2 × COCH); MS: $[M]^+$ at 444 (m/z); Analysis calculated for $C_{30}H_{24}O_2N_2$: C, 81.08; H, 5.41; N, 6.31. Found: C, 81.25; H, 5.70; N, 6.06.

General procedure for the synthesis of 4,4'-Bis-(*p*-methoxybenzylidenylacetyl)benzidine (3):

Compound 3 was prepared in a manner similar to that described for 2, starting from compound 1 (7.0 g, 0.026 mol) and *p*-methoxybenzaldehyde (3.18 ml,



Scheme 1: Synthesis of compounds 1-19.
Synthesis of compound with R=phenyl; *p*-methoxyphenyl; *p*-hydorxy-*m*-methoxyphenyl; *p*-*N,N*-dimethyliminophenyl; *p*-hydroxyphenyl; *o*-methoxyphenyl.

0.026 mol), in 65% (4.55 g) yield; MP (^{0}) 189-190; recrystallization solvent: ethanol; IR (KBr pellets) v cm^{-1}: 3285 (N-H); 3010 (C-H, aromatic), 2950 (C-H, aliphatic), 1660 (C=O), 1574 (C⋯C of aromatic ring), 1215 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 8.80 (s, 2H, 2 × NHCO, exchangeable with D_2O), 8.40 (d, J=7.0 Hz, 2H, 2 × Ar-CH), 7.05-7.80 (m, 16H, Ar-H), 3.40 (s, 6H, 2 × Ar-OCH_3), 6.51 (d, 2H, 2 × COCH, J=11.0 Hz); MS: $[M]^+$ at 504 (m/z); Analysis calculated for $C_{32}H_{28}O_4N_2$: C, 76.19; H, 5.56; N, 5.56. Found: C, 76.42; H, 5.80; N, 5.18.

4,4'-Bis-(*p*-hydroxy, *m*-methoxybenzylidenylacetyl) benzidine (4):
Compound 4 was prepared in a manner similar to that described for 2, starting from compound 1 (7.0 g, 0.026 mol) and *p*-hydroxy,*m*-methoxybenzaldehyde (3.97 g, 0.026 mol), in 68% (4.76 g) yield; MP (^{0}) 161-162; recrystallization solvent: ethanol; IR (KBr pellets) v cm^{-1}: 3277 (N-H), 3022 (C-H, aromatic), 2933 (C-H, aliphatic), 1566 (C⋯C of aromatic ring), 1668 (C=O), 1220 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 10.10 (s, 2H, 2 × OH, exchangeable with D_2O), 8.81 (s, 2H, 2 × NHCO, exchangeable with D_2O), 8.38 (d, J=7.0 Hz, 2H, 2 × Ar-CH), 7.77-7.06 (m, 14H, Ar-H), 6.49 (d, J=11.0 Hz, 2H, 2 × COCH), 3.42 (s, 6H, 2 × Ar-OCH_3); MS: $[M]^+$ at 536 (m/z); Analysis calculated for $C_{32}H_{28}O_6N_2$: C, 71.64; H, 5.22; N, 5.22. Found: C, 71.30; H, 5.52; N, 5.10.

4,4''-Bis-(*p-N,N*-dimethyliminobenzylidenylacetyl) benzidine (5):
Compound 5 was prepared in a manner similar to that described for 2, starting from compound 1 (7.0 g, 0.026 mol) and *p-N,N*-dimethyliminobenzaldehyde (3.90 g, 0.026 mol), in 60% (4.2 g) yield; MP (^{0}) 174-175; recrystallization solvent: DMF; IR (KBr pellets) v cm^{-1}: 3272 (N-H), 3017 (C-H, aromatic), 2938 (C-H, aliphatic), 1663 (C=O), 1572 (C⋯C of aromatic ring),1225 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 8.81 (s, 2H, 2 × NHCO, exchangeable with D_2O), 8.40 (d, J=7.0 Hz, 2H, 2 × Ar-CH), 7.82-7.01 (m, 16H, Ar-H), 6.53 (d, J=11.0 Hz, 2H, 2 × COCH), 2.13 (s, 12H, 2 × $N(CH_3)_2$); MS: $[M]^+$ at 530 (m/z); Analysis calculated for $C_{34}H_{34}O_2N_4$: C, 76.98; H, 6.42; N, 10.57. Found: C, 77.24; H, 6.15; N, 10.85.

4,4'-Bis-(*p*-hydroxybenzylidenylacetyl)benzidine (6):
Compound 6 was prepared in a manner similar to that described for 2, starting from compound 1 (7.0 g, 0.026 mol) and *p*-hydroxybenzaldehyde (3.19 g, 0.026 mol), in 60% yield, MP (^{0}) 157-158; recrystallization solvent: Methanol. IR (KBr pellets) v cm^{-1}: 3276 (N-H), 3028 (C-H, aromatic), 2940 (C-H, aliphatic), 1663 (C=O), 1575 (C⋯C of aromatic ring), 1221 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 10.14 (s, 2H, 2 × OH, exchangeable with D_2O), 8.81(s, 2H, 2 × NHCO, exchangeable with D_2O), 8.42 (d, J=7.0 Hz, 2H, 2 × Ar-CH), 7.78-7.04 (m, 16H, Ar-H), 6.49 (d, J=11.0 Hz, 2H, 2 × COCH); MS: $[M]^+$ at 476 (m/z); Analysis calculated for $C_{30}H_{24}O_4N_2$: C, 75.63; H, 5.04; N, 5.88. Found: C, 75.45; H, 5.40; N, 6.15.

4,4'-Bis-(*o*-methoxybenzylidenylacetyl)benzidine (7):
Compound 7 was prepared in a manner similar to that described for 2, starting from compound 1 (7.0 g, 0.026 mol) and o-methoxybenzaldehyde (3.56 g, 0.026 mol), in 63% (4.41 g) yield; MP (^{0}) 201-202; recrystallization solvent: Ethanol; IR (KBr pellets) v cm^{-1}: 3289 (N-H), 3018 (C-H, aromatic), 2953 (C-H aliphatic), 1665 (C=O), 1579 (C⋯C of aromatic ring), 1218 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 8.83 (s, 2H, 2 × NHCO, exchangeable with D_2O), 8.39 (d, J=7.0 Hz, 2H, 2 × Ar-CH), 7.82-7.05 (m, 16H, Ar-H), 6.51 (d, J=11.0 Hz, 2H, 2 × COCH), 3.44 (s, 6H, 2 × Ar-OCH_3); MS: $[M]^+$ at 504 (m/z); Analysis calculated for $C_{32}H_{28}O_4N_2$: C, 76.19; H, 5.56; N, 12.70. Found: C, 76.02; H, 5.30; N, 12.53.

General procedure for the synthesis of 4,4'-Bis-[1''-acetyl-5''-(phenyl)-2''-pyrazolin-3''-yl] benzidine (8):
To a solution of compound 2 (2.0 g, 0.0045 mol) in methanol (60 ml), hydrazine hydrate 99% (0.43 ml, 0.0090 mol) was added followed by 2 ml glacial acetic acid. Further, this reaction mixture was refluxed for 13 h. During post refluxing process, the remaining solvent was extracted in order to obtain solid matter and then it was poured onto pulverized ice, filtered, dried and finally recrystallized from methanol to give compound 8 (1.14 g, 57%) as colorless crystals; MP (^{0}) 224-225; IR (KBr pellets) v cm^{-1}: 3278 (N-H), 3027 (C-H, aromatic), 2858 (C-H of $COCH_3$), 1682 (C=O), 1600 (C=N), 1585 (C⋯C of aromatic ring), 1166 (C-N), 1022 (N-N); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 6.72 (t, J=5.0 Hz, 2H, 2 × CH-Ar of pyrazoline ring), 7.80-7.00 (m, 18H, Ar-H), 6.11 (s, 2H, 2 × NH, exchangeable with D_2O), 5.32 (d, J=9.0 Hz, 4H, 2 × CH_2 of pyrazoline ring), 2.22 (s, 6H, 2 × $COCH_3$); MS: $[M]^+$ at 532 (m/z); Analysis calculated for $C_{32}H_{32}O_2N_6$: C, 72.18; H, 6.06; N, 15.79. Found: C, 72.38; H, 5.88; N, 15.64.

4,4'-Bis-[1''-acetyl-5''-(*p*-methoxyphenyl)-2''-pyrazolin-3''-yl]benzidine (9):

Compound 9 was prepared in a manner similar to that described for 8, starting from compound 3 (2.0 g, 0.0040 mol) and hydrazine hydrate 99% (0.38 ml, 0.0080 mol), in 62% (1.24 g) yield; MP (^{0}) 177-178; recrystallization solvent: Ethanol; IR (KBr pellets) v cm^{-1}: 3283 (N-H), 3010 (C-H, aromatic), 2860 (C-H of $COCH_3$), 1680 (C=O), 1603 (C=N), 1580 (C⋯C of aromatic ring), 1167 (C-N), 1026 (N-N); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 7.82-7.08 (m, 16H, Ar-H), 6.75 (t, J=5.0 Hz, 2H, 2 × CH-Ar of pyrazoline ring), 6.14 (s, 2H, 2 × NH, exchangeable with D_2O), 5.30 (d, J=9.0 Hz, 4H, 2 × CH_2 of pyrazoline ring), 3.38 (s, 6H, 2 × Ar-OCH_3), 2.25 (s, 6H, 2 × $COCH_3$); MS: $[M]^+$ at 592 (m/z); Analysis calculated for $C_{34}H_{36}O_4N_6$: C, 68.92; H, 6.08; N, 14.19. Found: C, 68.70; H, 5.91; N, 14.24.

4,4'-Bis-[1''-acetyl-5''-(*p*-hydroxy, *m*-methoxyphenyl)-2''-pyrazolin-3''-yl] benzidine (10):

Compound 10 was prepared in a manner similar to that described for 8, starting from compound 4 (2.0 g, 0.0037 mol) and hydrazine hydrate 99% (0.36 ml, 0.0075 mol), in 60% (1.2 g) yield; MP (^{0}) 194-195; recrystallization solvent: acetic acid; IR (KBr pellets) v cm^{-1}: 3291 (N-H), 3023 (C-H, aromatic), 2857 (C-H of $COCH_3$), 1678 (C=O), 1602 (C=N), 1580 (C⋯C of aromatic ring), 1160 (C-N), 1020 (N-N); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 10.12 (s, 2H, 2 X OH, exchangeable with D_2O), 6.11 (s, 2H, 2 X NH, exchangeable with D_2O), 6.72 (t, J=5.0 Hz, 2H, 2 × CH-Ar of pyrazoline ring), 7.81-7.09 (m, 14H, Ar-H), 5.28 (d, J=9.0 Hz, 4H, 2 × CH_2 of pyrazoline ring), 3.41 (s, 6H, 2 × Ar-OCH_3), 2.27 (s, 6H, 2 × $COCH_3$); MS: $[M]^+$ at 624 (m/z); Analysis calculated for $C_{34}H_{36}O_6N_6$: C, 65.38; H, 5.77; N, 13.46. Found: C, 65.45; H, 5.85; N, 13.30.

4,4'-Bis-[1''-acetyl-5''-(*p*-*N*,*N*-dimethylimino-phenyl)-2''-pyrazolin-3''-yl]benzidine (11):

Compound 11 was prepared in a manner similar to that described for 8, starting from compound 5 (2.0 g, 0.0038 mol) and hydrazine hydrate 99% (0.36 ml, 0.0076 mol), in 56% (1.12 g) yield; MP (^{0}) 214-215; recrystallization solvent: DMF; IR (KBr pellets) v cm^{-1}: 3280 (N-H), 3020 (C-H, aromatic), 2863 (C-H of $COCH_3$), 1681 (C=O), 1604 (C=N), 1575 (C⋯C of aromatic ring), 1156 (C-N), 1030 (N-N); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 6.75 (t, J=5.0 Hz, 2H, 2 × CH-Ar of pyrazoline ring), 7.84-7.08 (m, 16H, Ar-H), 6.13 (s, 2H, 2 × NH, exchangeable with D_2O), 5.34 (d, J=9.0 Hz, 4H, 2 × CH_2 of pyrazoline ring), 2.26 (s, 6H, 2 × $COCH_3$), 2.12 (s, 12H, 2 × $N(CH_3)_2$); MS: $[M]^+$ at 618 (m/z); Analysis calculated for $C_{36}H_{42}O_2N_8$: C, 69.90; H, 6.80; N, 18.12. Found: C, 70.07; H, 6.71; N, 18.25.

4,4'-Bis-[1''-acetyl-5''-(*p*-hydroxyphenyl)-2''-pyrazolin-3''-yl]benzidine (12):

Compound 12 was prepared in a manner similar to that described for 8, starting from compound 6 (2.0 g, 0.0042 mol) and hydrazine hydrate 99% (0.40 ml, 0.0084 mol), in 55% (1.1 g) yield; MP (^{0}) 162-163; recrystallization solvent: ethanol; IR (KBr pellets) v cm^{-1}: 3281 (N-H), 3014 (C-H, aromatic), 2864 (C-H of $COCH_3$), 1682 (C=O), 1603 (C=N), 1588 (C⋯C of aromatic ring), 1167 (C-N), 1026 (N-N); ^{1}H NMR (DMSO-d_3, 300 MHz,) δ ppm: 10.08 (s, 2H, 2 × OH, exchangeable with D_2O), 7.81-7.10 (m, 16H, Ar-H), 6.72 (t, J=5.0 Hz, 2H, 2 × CH-Ar of pyrazoline ring), 6.08 (s, 2H, 2 × NH, exchangeable with D_2O), 5.30 (d, J=9.0 Hz, 4H, 2 × CH_2 of pyrazoline ring), 2.28 (s, 6H, 2 × $COCH_3$); MS: $[M]^+$ at 564 (m/z); Analysis calculated for $C_{32}H_{32}O_4N_6$: C, 68.09; H, 5.67; N, 14.89. Found: C, 68.18; H, 5.50; N, 14.72.

4,4'-Bis-[1''-acetyl-5''-(*o*-methoxyphenyl)-2''-pyrazolin-3''-yl]benzidine (13):

Compound 13 was prepared in a manner similar to that described for 8, starting from compound 7 (2.0 g, 0.0040 mol) and hydrazine hydrate 99% (0.38 ml, 0.0080 mol), in 58% (1.16 g) yield; MP (^{0}) 184-185; recrystallization solvent: ethanol; IR (KBr pellets) v cm^{-1}: 3282 (N-H), 3022 (C-H, aromatic), 2875 (C-H of $COCH_3$), 1673 (C=O), 1609 (C=N), 1570 (C⋯C of aromatic ring), 1166 (C-N), 1026 (N-N); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 6.74 (t, J=5.0 Hz, 2H, 2 × CH-Ar of pyrazoline ring), 7.82-7.10 (m, 16H, Ar-H), 6.10 (s, 2H, 2 × NH, exchangeable with D_2O), 5.28 (d, J=9.0 Hz, 4H, 2 × CH_2 of pyrazoline ring), 3.40 (s, 6H, 2 × Ar-OCH_3), 2.24 (s, 6H, 2 × $COCH_3$); MS: $[M]^+$ at 592 (m/z); Analysis calculated for $C_{34}H_{36}O_4N_6$: C, 68.92; H, 6.08; N, 14.19. Found: C, 69.10; H, 6.15; N, 14.01.

General procedure for the synthesis of 4,4'-Bis-[2''-(phenyl)-isoxazolin-4''-yl]benzidine (14):

To the solution of compound 2 (2.0 g, 0.0045 mol) in methanol (60 ml), hydroxylamine hydrochloride

(0.31 g, 0.0045 mol) was added in the presence of anhydrous NaOH (2.0 g). This mixture was refluxed for 8 h and then distilled off. To finally obtained to the recrystallized particles the remnants of reaction mixture passed through cooling process and then mingled with crushed ice. At later stage, the obtained substance filtered and dried up to get the final product. In continuity the final extracted product was recrystallized from DMF to furnish compound 14 (1.04 g, 52%) as pale yellow crystals; MP (^{0}) 239-240; IR (KBr pellets) v cm^{-1}: 3289 (N-H), 3022 (C-H, aromatic), 1605 (C=N), 1570 (C⋯C of aromatic ring); 1252 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 6.66 (t, 2H, J=5.0 Hz, 2 × CH-Ar of isoxazoline ring), 7.94-7.10 (m, 18H, Ar-H), 6.23 (s, 2H, 2 × NH, exchangeable with D_2O), 5.30 (d, J=6.0 Hz, 4H, 2 × CH_2 of isoxazoline ring); MS: $[M]^+$ at 474 (m/z); Analysis calculated for $C_{30}H_{26}O_2N_4$: C, 75.95; H, 5.49; N, 11.81. Found: C, 75.74; H, 5.70; N, 11.62.

4,4'-Bis-[2''-(*p*-methoxyphenyl)-isoxazolin-4''-yl] benzidine (15):

Compound 15 was prepared in a manner similar to that described for 14, starting from compound 3 (2.0 g, 0.004 mol) and hydroxylamine hydrochloride (0.28 g, 0.004 mol), in 55% (1.10 g) yield; MP (^{0}) 220-221; recrystallization solvent: ethanol; IR (KBr pellets) v, cm^{-1}: 3284 (N-H), 3021 (C-H, aromatic), 1602 (C=N), 1570 (C⋯C of aromatic ring), 1250 (C-O-C); ^{1}H NMR (DMSO-d_3, 300 MHz,) δ ppm: 6.65 (t, 2H, J=5.0 Hz, 2 × CH-Ar of isoxazoline ring), 7.91-7.12 (m, 16H, Ar-H), 6.25 (s, 2H, 2 × NH, exchangeable with D_2O), 5.30 (d, J=6.0 Hz, 4H, 2 × CH_2 of isoxazoline ring), 3.45 (s, 6H, 2 × Ar-OCH_3); MS: $[M]^+$ at 534 (m/z); Analysis calculated for $C_{32}H_{30}O_4N_4$: C, 71.91; H, 5.62; N, 10.49. Found: C, 71.58; H, 5.35; N, 10.74.

4,4'-Bis-(2''-(*p*-hydroxy,*m*-methoxyphenyl)-isoxazolin-4''-yl)benzidine (16):

Compound 16 was prepared in a manner similar to that described for 14, starting from compound 4 (2.0 g, 0.0037 mol) and hydroxylamine hydrochloride (0.26 g, 0.0037 mol), in 53% (1.06 g) yield; MP (^{0}) 250-251; recrystallization solvent: ethanol; IR (KBr pellets) v cm^{-1}: 3286 (N-H), 3028 (C-H, aromatic), 1602 (C=N), 1571 (C⋯C of aromatic ring), 1248 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 10.11 (s, 2H, 2 × OH, exchangeable with D_2O), 6.64 (t, 2H, J=5.0 Hz, 2 × CH-Ar of isoxazoline ring), 6.22 (s, 2H, 2 × NH, exchangeable with D_2O), 7.89-7.15 (m, 14H, Ar-H), 5.28 (d, J=6.0 Hz, 4H, 2 × CH_2 of isoxazoline ring), 3.40 (s, 6H, 2 × Ar-OCH_3); MS: $[M]^+$ at 566; Analysis calculated for $C_{32}H_{30}O_6N_4$: C, 67.84; H, 5.30; N, 9.89. Found: C, 67.60; H, 5.52; N, 9.60.

4,4'-Bis-[2''-(*p*-*N*,*N*-dimethylimino-phenyl)-isoxazolin-4''-yl)]benzidine (17):

Compound 17 was prepared in a manner similar to that described for 14, starting from compound 5 (2.0 g, 0.0038 mol) and hydroxylamine hydrochloride (0.26 g, 0.0038 mol), in 50% (1.0 g) yield; MP (^{0}) 264-265; recrystallization solvent: ethanol; IR (KBr pellets) v cm^{-1}: 3283 (N-H), 3018 (C-H, aromatic), 1600 (C=N), 1577 (C⋯C of aromatic ring), 1262 (C-O-C); ^{1}H NMR ($CDCl_3$, 300 MHz,) δ ppm: 7.95-7.10 (m, 16H, Ar-H), 6.62 (t, J=5.0 Hz, 2H, 2 × CH-Ar of isoxazoline ring), 6.27 (s, 2H, 2 × NH, exchangeable with D_2O), 5.33 (d, J=6.0 Hz, 4H, 2 × CH_2 of isoxazoline ring), 2.15 (s, 12H, 2 × $N(CH_3)_2$); MS: $[M]^+$ at (m/z) 560; Analysis calculated for $C_{34}H_{36}O_2N_6$: C, 72.86; H, 6.43; N, 15.00. Found: C, 72.58; H, 6.63; N, 15.27.

4,4'-Bis-[2''-(*p*-hydroxyphenyl)-isoxazolin-4''-yl] benzidine (18):

Compound 18 was prepared in a manner similar to that described for 14, starting from compound 6 (2.0 g, 0.0042 mol) and hydroxylamine hydrochloride (0.29 g, 0.042 mol), in 51% (1.02 g) yield; MP (^{0}) 244-245; recrystallization solvent: methanol; IR (KBr pellets) v cm^{-1}: 3279 (N-H), 3020 (C-H, aromatic), 1601 (C=N), 1564 (C⋯C of aromatic ring), 1243 (C-O-C); ^{1}H NMR (DMSO-d_6, 300 MHz,) δ ppm: 10.10 (s, 2H, 2 × OH, exchangeable with D_2O), 6.62 (t, 2H, J=5.0 Hz, 2 × CH-Ar of isoxazoline ring), 6.25 (s, 2H, 2 × NH, exchangeable with D_2O), 7.90-7.14 (m, 16H, Ar-H), 5.32 (d, J=6.0 Hz, 4H, 2 × CH_2 of isoxazoline ring); MS: $[M]^+$ at 506 (m/z); Analysis calculated for $C_{30}H_{26}O_4N_4$: C, 71.15; H, 5.14; N, 11.07. Found: C, 71.40; H, 5.45; N, 11.30.

4,4'-Bis-[2''-(*o*-methoxyphenyl)-isoxazolin-4''-yl] benzidine (19):

Compound 19 was prepared in a manner similar to that described for 14, starting from compound 7 (2.0 g, 0.004 mol) and hydroxylamine hydrochloride (0.28 g, 0.004 mol), in 54% (1.08 g) yield; MP (^{0}) 230-231; recrystallization solvent: ethanol; IR (KBr pellets) v cm^{-1}: 3284 (N-H), 3022 (C-H, aromatic), 1603 (C=N), 1570 (C⋯C of aromatic ring), 1250

(C-O-C); ^{1}H NMR (DMSO-d_6, 300 MHz,) δ ppm: 6.62 (t, 2H, J=5.0 Hz, 2 × CH-Ar of isoxazoline ring), 6.23 (s, 2H, 2 × NH, exchangeable with D_2O), 7.91-7.13 (m, 16H, Ar-H), 5.28 (d, J=6.0 Hz, 4H, 2 × CH_2 of isoxazoline ring), 3.40 (s, 6H, 2 × Ar-OCH_3); MS: $[M]^+$ at 534 (m/z); Analysis calculated for $C_{32}H_{30}O_4N_4$: C, 71.91; H, 5.62; N, 10.49. Found: C, 71.65; H, 5.41; N, 10.35.

Pharmacological evaluation:

The compounds 2-19 were evaluated for their antiinflammatory activity and acute toxicity. Most promising compounds of the present study were also assessed for their ulcerogenic profile. Phenylbutazone (CAS 50-33-9) (from commercial source), potent antiinflammatory drug, was used as reference drug. The antiinflammatory and ulcerogenic activities were conducted on albino rats of Charles Foster strain, 80 to 110 days old weighing around 80-120 g. Acute toxicity profile was tested on albino mice (25-30 g). The animals were maintained at the following conditions: 25±2° temperature, 50±2% relative humidity, 12 h light/dark cycle, food and tap water *ad libitum*. The Ethics Committee of L. L. R. M. Medical College, Meerut, India, approved the care and use of animals and the experimental protocols used in this study.

Acute toxicity study:

Acute toxicity of compounds 2-19 and phenylbutazone were evaluated by employing the process of Smith[10]. Test drugs were administered at various doses in batches of albino mice. Percent mortality was observed in every batch of animals after drug administration. With the help of obtained data, ALD_{50} was measured.

Antiinflammatory activity:

Compounds 2-19 were screened *in vivo* for their antiinflammatory activity by adopting the standard procedure[11]. Then, segregation of albino rats was done into control, drug treated and standard drug groups. Each group had six animals. Then, suspension of carrageenan was prepared, i.e. 1% in 0.9% saline. This freshly prepared suspension was injected (0.05 ml) at right hind paw of every rat. Before one hour of carrageenan injection, albino rats of drug treated and standard drug groups were treated with test compound (2-19) and phenylbutazone, respectively. And, animals of control group were treated with propylene glycol. With the help of Plethysmometer, paw volume of each rat was assessed at two stages i.e. before one hour and three hours after carrageenan injection. Antiinflammatory activity was expressed as a percentage calculated using the formula, % antiinflammatory activity=$(1-PV_t/PV_c)\times100$, where PV_t is the mean increase in paw volume of drug treated group; PV_c is the mean increase in paw volume of control group.

Ulcerogenic activity:

The ulcerogenic liability of compounds (9 and 15) and standard drug (phenylbutazone) was checked on albino rats according to standard procedure[12]. The rats were deprived of food for 24 h before the administration of the drug. Animals were given water *ad libitum*. The standard drug and compounds (9 and 15) were given orally to the different groups of animals. After eight hours of drug treatment, animals were sacrificed. The duodenum, jejunum and stomach were extracted, and then studied for any of the evidences of the following: (i) petechial or frank hemorrhage, (ii) shedding of epithelium, (iii) discrete or erosion ulceration. Existence of any of the aforesaid proof was marked as an impression of ulcerogenic liability.

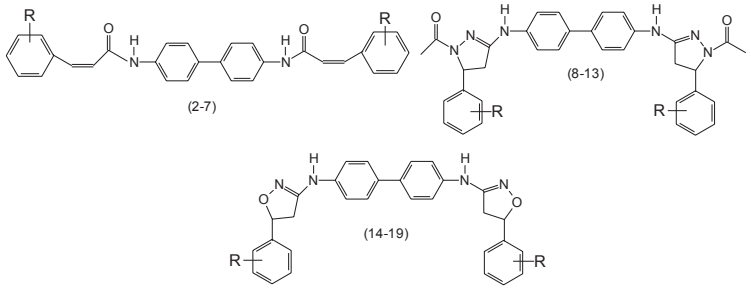
Cyclooxygenase assay:

The objective of this method was to explore the most possible mechanism of action of compound. The test was executed *in vitro* focusing on the microsomal fraction of mucosal preparations of rabbit distal colon according to procedure of Calderano *et al.*[13]. Percent inhibition of cyclooxygenase enzyme was calculated.

Lipophilicity determination:

Lipophilicity is possibly the most important physicochemical property of a potential drug, it plays a role in solubility, absorption, membrane penetration, plasma protein binding, distribution, CNS penetration and partitioning into other tissues or organs such as the liver and has an impact on the routes of clearance. In order to determine the pharmacological potential of compounds 2-19, these compounds were subjected to lipophilicity determination theoretically as well as experimentally. The theoretically lipophilicity (log P) was calculated using the freely accessible Molinspiration Cheminformatics Software (www.molinspiration.com; Slovensky Grob, Slovak Republic). The log P values of compounds 2-19 were determined experimentally by following procedure: before each determination, the purity of the

TABLE 1: PHARMACOLOGICAL DATA OF COMPOUNDS 2-19



Compounds	R	Acute Toxicity ALD_{50} (mg/kg p.o.)	Antiinflammatory activity		Ulcerogenic activity UD_{50} (mg/kg p.o.)	Cyclooxygenase assay (% inhibition 10 µM)	Lipophilicity	
			Dose (mg/kg p.o.)	% inhibition of oedema			log P_{cal}	log P_{exp}
Control		-		0.0	-	-	-	-
Phenylbutazone		>280	25	26.50*	66.6	90	-	-
			50	44.60*				
			100	65.10*				
2	H	>800	50	16.32*	-	-	6.792	6.510
3	*ρ*-OCH_3	>800	50	35.12*	-	-	6.905	6.701
4	*ρ*-OH, m-OCH_3	>800	50	27.20*	-	-	5.47	5.125
5	*ρ*-$N(CH_3)_2$	>800	50	15.23*	-	-	6.997	6.710
6	*ρ*-OH	>800	50	19.41*	-	-	5.834	5.417
7	*o*-OCH_3	>800	50	29.20*	-	-	6.45	6.101
8	-H	>800	50	23.30*	-	-	6.649	6.419
9	*ρ*-OCH_3	>1600	25	30.30*	162.2	75	6.762	6.358
			50	54.80*				
			100	69.20*				
10	*ρ*-OH, m-OCH_3	>800	50	38.17*	-	-	5.327	5.246
11	*ρ*-$N(CH_3)_2$	>800	50	25.40*	-	-	6.854	6.579
12	*ρ*-OH	>800	50	27.20*	-	-	5.691	5.428
13	*o*-OCH_3	>800	50	42.20*	-	-	6.666	6.780
14	-H	>800	50	20.30*	-	-	6.64	6.601
15	*ρ*-OCH_3	>800	25	27.80*	130.6	79	6.754	6.158
			50	50.70*				
			100	67.32*				
16	*ρ*-OH, m-OCH_3	>800	50	35.52*	-	-	5.318	5.024
17	*ρ*-$N(CH_3)_2$	>800	50	22.3*	-	-	6.845	6.428
18	*ρ*-OH	>800	50	24.34*	-	-	5.682	5.412
19	*o*-OCH_3	>800	50	39.36*	-	-	6.658	6.015

n=6; **P*<0.05 assessed with control; propylene glycol served as control; - denotes that activity was not performed; All log P_{exp} are obtained by UV/Vis

compounds was checked by TLC using two pairs of eluents. Let us recall only that log P was calculated the decimal logarithm of the ratio of the solute concentration in *n*-octanol and in water after partition equilibrium. An octanol solution (saturated in water) of a solute 10 ml was introduced into a 250 ml separating funnel with 50 ml of water (previously saturated in *n*-octanol). It was stirred in a mechanical shaker for 0.5 h. The solutions were then left to stand for 24 h until the two phases were separated. At equilibrium, the aqueous solution separated then the concentration of solute is determined by UV/Vis spectrophotometer.

RESULTS AND DISCUSSION

All compounds (2-8 and 10-19) of the present study showed ALD_{50} >800 mg/kg p.o., except compound 9 exhibiting >1600 mg/kg p.o. (Table 1). The standard drug was found to possess ALD_{50}>280 mg/kg p.o. Eighteen newly synthesized compounds 2-19 along with reference drugs, phenylbutazone were studied

for antiinflammatory activity at a dose of 50 mg/kg per oral against carrageenan induced odema. All the compounds of this series have shown statistically significant antiinflammatory activity of moderate to potent degree (15.23 to 54.80%) (Table 1). It was also noticed that two compounds 9 and 15 exhibited maximum inflammation inhibiting activity (54.80 and 50.70%, respectively) than phenylbutazone (44.60%). Having observed the potentialities of compounds 9 and 15, we were motivated to assess both compounds with phenylbutazone to study antiinflammatory activity with two additional doses i.e. 25 and 100 mg/kg p.o. Results indicate that compounds 9 and 15 displayed substantial inflammation inhibiting property when compared to standard drug (Table 1).

Ulcerogenic liability of compounds 9, 15 and phenylbutazone were tested. The UD_{50} of compounds 9, 15 and phenylbutazone are 162.2, 130.6 and 66.6 mg/kg p. o., respectively. In order to explore the most possible mechanism of action of compounds 9, 15 and phenylbutazone (standard drug) were subjected to screen for cyclooxygenase assay for obstructing the odema formation. Obtained results suggested that inflammation was reduced by lowering the prostaglandin (PG) synthesis by inhibiting the cyclooxygenase (COX) enzyme.

Results showed that substituted benzylidenes (2-7) exhibited mild to moderate antiinflammatory activity (16.23, 35.12, 27.2, 15.23, 19.41 and 29.20%, respectively). It is, also, significant to mention that conversion of compounds 2-7 into their corresponding pyrazoline (8-13: 23.30, 54.80, 38.17, 25.40, 27.20 and 42.20%, respectively) and isoxazoline (14-19: 20.30, 50.70, 35.52, 22.30, 24.34 and 39.36%, respectively) congeners enhanced the antiinflammatory activity. However, pyrazolines (8-13) possessed better antiinflammatory profile than isoxazolines (14-19).

By assessing the effects of certain substituting groups like *p*-methoxyphenyl, phenyl, *p*-hydorxy-*m*-methoxyphenyl, *p*-aminodimethylphenyl, *p*-hydroxyphenyl and *o*-methoxy phenyl as depicted in Table 1, it was observed that compounds 2, 8 and 14 having phenyl group as substitutent showed least percent inhibition of oedema (16.32, 23.30 and 20.30%, respectively), whereas substitution with *p*-methoxyphenyl group as seen in compounds 3, 9 and 15 exhibited maximal antiinflammatory activity (35.12, 54.80 and 50.70%, respectively). Furthermore, *o*-methoxyphenyl substitution in compounds 7, 13 and 19 displayed less but adequate antiinflammatory activity (29.20, 42.20 and 39.36%, respectively). Hence, it would be interesting to point out that presence of methoxy group as a substituent either at *o*- or *m*- or *p*-position of phenyl group in different compounds showed substantive antiinflammatory activity.

Out of six pyrazoline congeners, compound 4,4'-bis-[1''-acetyl-5''-(*p*-methoxy phenyl)-2''-pyrazolin-3''-yl] benzidine (9) was found to be the most potent compound of the present study and this compound possessed ALD_{50}>1600 mg/kg suggesting good safety margin. Moreover, this compound was also tested for its ulcerogenic liability, and was found to be less ulcerogenic (UD_{50}=162.2 mg/kg). Results also indicated the correlation between the percent odema inhibition by synthesized compounds 2-19 and lipophilicity theoretically and experimentally determining log P values. Higher activity corresponds to high log P values.

From the results and discussion, it is thereby concluded that substituted benzylidene congeners exhibit mild to moderate antiinflammatory profile; cyclization of these substituted benzylidenes into their corresponding pyrazoline and isoxazoline congeners enhances the inflammation inhibiting property; pyrazoline derivatives possess better antiinflammatory activity than isoxazoline congeners; presence of methoxy group either at *o*- or *m*- or *p*-position of phenyl ring as a substituent elicits a remarkable increase in antiinflammatory activity.

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