



Synthesis and Biological Activity of 2-(Thiazolidin-4-One) Phenyl]-1h-Phenylbenzimidazoles and 2-[4-(Azetidin-2-One)-3-Chloro-4- Phenyl] - 1h-Phenyl Benzimidazoles

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ABSTRACT

A new series of 2-[4-(azetidin-2-one)-3-Chloro-4-phenyl]-1H-Phenylbenzimidazoles and 2-(thiazolidin-4-one)-Phenyl]-1H-Phenylbenzimidazoles were synthesized by the reaction of schiff base [2-(4-aminophenyl)-Benzimidazole and substituted Benzaldehyde] with chloroacetyl chloride and mercaptoacetic acid respectively. The chemical structures of the synthesized compounds were confirmed by IR, ¹H-NMR, mass spectral and C, H, N analysis. The synthesized compounds were screened for antibacterial (*Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella epidermidis*), antifungal (*Aspergillus niger* and *Candida albicans*), analgesic activity by writhing reflex method and anti-inflammatory activity by carrageenan induced paw edema method. The synthesized compound showed significant activity of antibacterial, antifungal, analgesic and anti-inflammatory activity comparable to that of standard.

Keywords: Azetidinone, Thiazolidinone, Benzimidazole, Analgesic, Anti-inflammatory, Antibacterial, Antifungal.

INTRODUCTION

Azetidinone and Thiazolidinone derivatives were reported to possess antibacterial, antifungal [1-2], antitubercular activity [3], anti-HIV [4], analgesic, anti inflammatory [5] and ulcerogenic activity. [6] Benzimidazole derivatives were reported to possess antibacterial [7], antifungal [8], anti-inflammatory [8], antiviral [9], antitumor [9], antioxidant [10] and antihelmintic [11] activities. Therefore it was envisaged that compounds containing both the chemical moieties would result in compounds of interesting biological activities. In this present study 2-(4-aminophenyl)-benzimidazole [12] were treated with different substituted aromatic aldehydes to produce Schiff base. [13-14] The Schiff bases were subjected to addition reactions with chloroacetyl chloride in the presence

of triethylamine and thioglycolic acid in the presence of 1, 4dioxane-anhydrous zinc chloride to produce 2-azetidinone derivatives and 4-thiazolidinone derivatives respectively [15-16] was prepared as per Fig. 1. The chemical structures of the synthesized compounds were confirmed by IR, ¹H-NMR, mass spectral and elemental analysis. The synthesized compounds were screened for antibacterial (*Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella epidermidis*), antifungal (*Aspergillus niger* and *Candida albicans*), analgesic activity by writhing reflex method and anti-inflammatory activity by carrageenan induced paw edema method.

MATERIAL METHODS

CHEMISTRY

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FTIR spectrometer MB104 with KBr

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pellets. $^1\text{H-NMR}$ spectra were recorded on 400 MHz-Joel GSX 400 using CDCl_3 as solvent. The chemical shifts are reported as parts per million downfield from tetra methyl silane (Me_4Si). Mass spectra were recorded on Shimadzu GC-MS QP 5000. Microanalyses for C, H and N were performed in Heraeus CHN Rapid Analyzer and analyses indicated by the symbols of the elements are within $\pm 0.4\%$ of the theoretical values. $^1\text{H-NMR}$ and IR spectra were consistent with the assigned structures. The purity of the compounds was checked by TLC on pre-coated aluminum sheets (Silica gel 60 F₂₅₄ Merck-Germany) using (2:4) ethyl acetate: hexane and (3:4) petroleum ether (40-60°C): ethyl acetate as mobile phase and visualized by iodine vapors.

General Method for the Synthesis of Schiff base (Ia-h)

A mixture of 2-(4-aminophenyl) Benzimidazole (0.01 mol), substituted Benzaldehyde (0.01 mol) and a drop of acetic acid was dissolved in ethanol (25 ml) and heated on a steam bath for 45-60 min. The reaction mixture was allowed to stand at room temperature for 24 h. The product separated out was filtered, dried under vacuum and recrystallized by using warm ethanol. The synthetic scheme of the title compounds are outlined in Scheme 1.

Synthesis of 2-[4-(azetidin-2-one) 3-Chloro-4-phenyl]-1H-Phenylbenzimidazole (A₁₋₈)

Chloroacetyl chloride (0.01mol) was added drop wise to a mixture of schiff base (0.01mol) and triethylamine (0.02 mol) in dioxane (25 ml) at room temperature. The mixture was stirred for 8 h and allowed to stand at room temperature for 3 days. The contents were poured on crushed ice and the precipitate obtained was filtered, washed with 10 % w/v sodium bicarbonate solution, vacuum dried and recrystallised using absolute ethanol.

Spectral data of synthesized compounds

Compound A₁: Yield = 48%, mp 250-252 °C, R_f = 0.54, $^1\text{H-NMR}$ (CDCl_3) δ : 7.95 (m, 4H; B_Z), 7.73 (m, 4H; Ph), 7.30-7.46 (m, 5H; Ph), 4.49 (s, 1H; 4-CH), 3.57 (s, 1H; 3-CH). IR (KBr) cm^{-1} : 3506, 3384, 3048, 1694, 1269, 1176, 865, 808, 731, 562, 541. EI-MS m/z : 373.74 (Calcd for $\text{C}_{22}\text{H}_{16}\text{N}_3\text{ClO}$: 373.82) Anal. Calcd for $\text{C}_{22}\text{H}_{16}\text{N}_3\text{ClO}$: C, 61.05; H, 3.52; N, 8.90. Found: C, 61.12; H, 3.32; N, 8.78.

Compound A₂: Yield = 44%, mp 230-232 °C, R_f = 0.58, $^1\text{H-NMR}$ (CDCl_3) δ : 7.94 (m, 4H; B_Z), 7.73 (m, 4H; Ph), 7.29-7.57 (dd, 4H; Ph), 4.49 (s, 1H; 4-CH), 3.06 (s, 1H; 3-CH). IR (KBr) cm^{-1} : 3507, 3379, 3048, 1694, 1269, 1177, 865, 809, 775, 731, 562, 506. EI-MS m/z : 408.18 (Calcd for $\text{C}_{22}\text{H}_{15}\text{N}_3\text{Cl}_2\text{O}$: 408.26) Anal. Calcd for: C, 55.03; H, 2.89; N, 8.02. Found: C, 55.16; H, 2.99; N, 8.10.

Compound A₃: Yield = 38%, mp 258-260 °C, R_f = 0.54, $^1\text{H-NMR}$ (CDCl_3) δ : 7.95 (m, 4H; B_Z), 7.73 (m, 4H; Ph), 7.07-7.45 (m, 4H; Ph), 4.49 (s, 1H; 4-CH), 3.57 (s, 1H; 3-CH). IR (KBr) cm^{-1} : 3507, 3064, 1696, 1267, 1116, 876, 758, 749, 724, 575, 507. EI-MS m/z : 389.76 (Calcd for $\text{C}_{22}\text{H}_{16}\text{N}_3\text{ClO}_2$: 389.82). Anal. Calcd for: C, 55.03; H, 2.89; N, 8.02. Found: C, 55.16; H, 2.98; N, 8.09.

Compound A₄: Yield = 54%, mp 248-250 °C, R_f = 0.71, $^1\text{H-NMR}$ (CDCl_3) δ : 7.92 (m, 4H; B_Z), 7.74 (m, 4H; Ph), 7.30-7.46 (m, 4H; Ph), 4.48 (s, 1H; 4-CH), 3.61 (s, 1H; 3-CH), 1.61 (s, 3H; CH₃). IR (KBr) cm^{-1} : 3507, 3380, 3048, 1694, 1269, 1176, 865, 808, 776, 562, 541. EI-MS m/z : 387.72 (Calcd for $\text{C}_{23}\text{H}_{18}\text{N}_3\text{ClO}$: 387.85). Anal. Calcd for: C, 53.41; H, 2.80; N, 11.70. Found: C, 53.52; H, 3.01; N, 11.92.

Compound A₅: Yield = 16%, mp 274-276 °C, R_f = 0.80, $^1\text{H-NMR}$ (CDCl_3) δ : 7.95 (m, 4H; B_Z), 7.73 (m, 4H; Ph), 7.30-

7.45 (m, 4H; Ph), 4.49 (s, 1H; 4-CH), 3.57 (s, 1H; 3-CH), 2.98 (s, 3H; 4-N-CH₃), 2.79 (s, 3H; 4-N-CH₃). IR (KBr) cm^{-1} : 3507, 3375, 3047, 1695, 1269, 1176, 865, 808, 776, 562, 541. EI-MS m/z : 416.78 (Calcd for $\text{C}_{24}\text{H}_{21}\text{N}_4\text{ClO}$: 416.89). Anal. Calcd for: C, 53.41; H, 2.80; N, 11.70. Found: C, 53.56; H, 2.71; N, 11.88.

Compound A₆: Yield = 61%, mp 220 °C, R_f = 0.56, $^1\text{H-NMR}$ (CDCl_3) δ : 7.97 (m, 4H; B_Z), 7.72 (m, 4H; Ph), 7.27-7.45 (m, 4H; Ph), 4.10 (s, 1H; 4-CH), 3.89 (s, 1H; 3-CH), 2.18 (s, 3H; 4-OCH₃). IR (KBr) cm^{-1} : 3468, 3067, 1689, 833, 754, 702, 614. EI-MS m/z : 403.76 (Calcd for $\text{C}_{23}\text{H}_{18}\text{N}_3\text{ClO}_2$: 403.85). Anal. Calcd for : C, 53.41; H, 2.80; N, 11.70. Found: C, 53.54; H, 2.66; N, 11.82.

Compound A₇: Yield = 46%, mp 238-240 °C, R_f = 0.88, $^1\text{H-NMR}$ (CDCl_3) δ : 7.94 (m, 4H; B_Z), 7.72 (m, 4H; Ph), 7.30-7.45 (m, 3H; Ph), 4.49 (s, 1H; 4-CH), 3.57 (s, 1H; 3-CH), 2.09 (s, 3H; 4-OCH₃), 1.84 (s, 3H; 3-OCH₃). IR (KBr) cm^{-1} : 3507, 3382, 1694, 1269, 1176, 865, 808, 776, 562. EI-MS m/z : 434.76 (Calcd for $\text{C}_{24}\text{H}_{20}\text{N}_3\text{ClO}_3$: 434.88). Anal. Calcd for: C, 62.10; H, 3.98; N, 8.52. Found: C, 62.24; H, 3.81; N, 8.39.

Compound A₈: Yield = 10%, mp 230-232 °C, R_f = 0.72, $^1\text{H-NMR}$ (CDCl_3) δ : 7.95 (m, 4H; B_Z), 7.73 (m, 4H; Ph), 7.23-7.45 (m, 2H; Ph), 4.49 (s, 1H; 4-CH), 3.90 (s, 1H; 3-CH), 2.28 (s, 3H; 3-OCH₃), 2.09 (s, 3H; 4-OCH₃), 1.82 (s, 3H; 5-OCH₃). IR (KBr) cm^{-1} : 3507, 3385, 3047, 1695, 1269, 1177, 865, 809, 776, 526. EI-MS m/z : 465.78 (Calcd for $\text{C}_{25}\text{H}_{22}\text{N}_3\text{ClO}_4$: 465.92). Anal. Calcd for: C, 60.41; H, 4.51; N, 11.74. Found: C, 60.58; H, 4.38; N, 11.62.

Synthesis of 2-[2-(thiazolidin-4-one) Phenyl]-1H-Phenylbenzimidazoles (T₁₋₈)

To a mixture of schiff base (0.01 mol) and mercaptoacetic acid (0.01 mol) dissolved in dioxane (20 ml), anhydrous zinc chloride (0.004 mol) was added and refluxed for 8 h. The reaction mixture was cooled, filtered, washed with 10 % w/v sodium bicarbonate solution, vacuum dried and recrystallised using absolute ethanol.

Compound T₁: Yield = 64%, mp 208-210 °C, R_f = 0.57, $^1\text{H-NMR}$ (CDCl_3) δ : 8.02 (m, 4H; B_Z), 7.65 (m, 4H; Ph), 6.90-7.44 (m, 5H; Ph), 4.15 (d, J = 2.1 Hz, 1H; 2-CH), 2.49 (s, 2H; 5-CH₂). IR (KBr) cm^{-1} : 3183, 1696, 1269, 1116, 783, 757, 748, 638. EI-MS m/z : 371.32 (Calcd for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{OS}$: 371.44). Anal. Calcd for: C, 71.13; H, 4.63; N, 11.32. Found: C, 71.12; H, 4.59; N, 11.28.

Compound T₂: Yield = 55%, mp 198-200 °C, R_f = 0.62, $^1\text{H-NMR}$ (CDCl_3) δ : 8.01 (m, 4H; B_Z), 7.64 (m, 4H; Ph), 6.90-7.45 (dd, J = 2.1 Hz, 4H; Ph), 4.15 (d, J = 2.2 Hz, 1H; 2-CH), 2.47 (s, 2H; 5-CH₂). IR (KBr) cm^{-1} : 3182, 1693, 1228, 748, 751, 723, 641, 503. EI-MS m/z : 405.78 (Calcd for $\text{C}_{22}\text{H}_{16}\text{N}_3\text{ClOS}$: 405.89). Anal. Calcd for: C, 65.08; H, 3.97; N, 10.35. Found: C, 64.98; H, 3.93; N, 10.28.

Compound T₃: Yield = 87%, mp 258-260 °C, R_f = 0.88, $^1\text{H-NMR}$ (CDCl_3) δ : 8.01 (m, 4H; B_Z), 7.62 (m, 4H; Ph), 6.98-7.45 (m, 4H; Ph), 4.15 (d, J = 2.1 Hz, 1H; 2-CH), 2.49 (s, 2H; 5-CH₂). IR (KBr) cm^{-1} : 3185, 1704, 1228, 784, 747, 721, 640, 504. EI-MS m/z : 387.26 (Calcd for: 387.45). Anal. Calcd for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$: C, 68.92; H, 4.42; N, 10.85. Found: C, 69.08; H, 4.38; N, 10.83.

Compound T₄: Yield = 98%, mp 278-280 °C, R_f = 0.57, $^1\text{H-NMR}$ (CDCl_3) δ : 8.09 (m, 4H; B_Z), 7.63 (m, 4H; Ph), 6.97-7.46 (m, 4H; Ph), 4.14 (d, J = 2.1 Hz, 1H; 2-CH), 2.36 (s, 2H; 5-CH₂), 1.59 (s, 3H; CH₃). IR (KBr) cm^{-1} : 3186, 1712, 1229, 1115, 783, 747, 723, 639. EI-MS m/z : 385.36 (Calcd

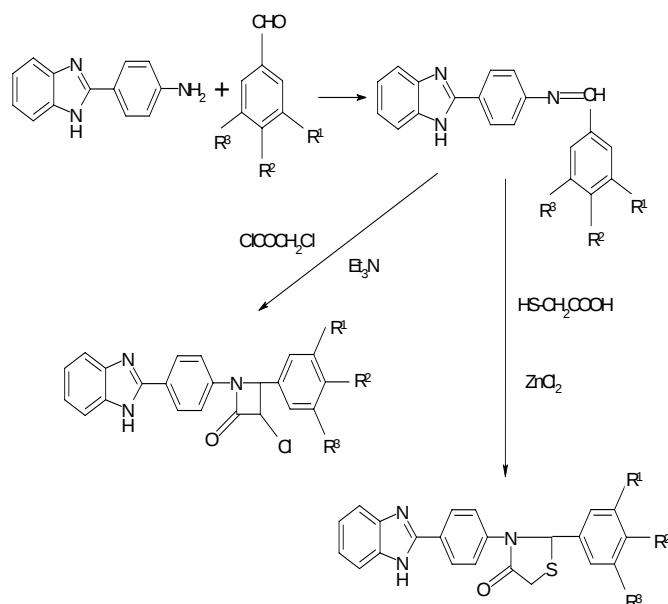
for: 385.45). *Anal.* Calcd for $C_{23}H_{19}N_3OS$: C, 71.66; H, 4.97; N, 10.90. Found: C, 71.61; H, 4.85; N, 10.90.

Compound T₅: Yield = 84%, mp 208-210 °C, *R_f* = 0.55, 1H -NMR ($CDCl_3$) δ : 8.22 (m, 4H; B_Z), 7.85 (m, 4H; Ph), 6.96-7.69 (m, 4H; Ph), 4.19 (d, *J* = 2.1 Hz, 1H; 2-CH), 2.51 (s, 2H; 5-CH₂), 2.16 (s, 3H; NCH₃), 1.85 (s, 3H; NCH₃). IR (KBr) cm^{-1} : 3137, 1711, 1229, 1113, 784, 745, 720, 638. EI-MS *m/z*: 414.36 (Calcd for $C_{24}H_{22}N_4OS$: 414.52). *Anal.* Calcd for: C, 69.54; H, 5.35; N, 13.52. Found: C, 69.50; H, 5.29; N, 13.50.

Compound T₆: Yield = 84%, mp 258-260 °C, *R_f* = 0.6, 1H -NMR ($CDCl_3$) δ : 8.01(m, 4H; B_Z), 7.65 (m, 4H; Ph), 6.90-7.44 (m, 4H; Ph), 4.15 (d, *J* = 2.1 Hz, 1H; 2-CH), 2.49 (s, 2H; 5-CH₂), 2.28 (s, 3H; OCH₃). IR (KBr) cm^{-1} : 3183, 1721, 1229, 1111, 784, 743, 722, 640. EI-MS *m/z*: 401.38 (Calcd for: 401.47). *Anal.* Calcd for $C_{23}H_{19}N_3O_2S$: C, 68.80; H, 4.77; N, 10.47. Found: C, 68.75; H, 4.74; N, 10.39.

Compound T₇: Yield = 82%, mp 278-280 °C, *R_f* = 0.43, 1H -NMR ($CDCl_3$) δ : 8.01(m, 4H; B_Z), 7.89 (m, 4H; Ph), 6.96-7.65 (m, 3H; Ph), 4.15 (d, *J* = 2.1 Hz, 1H; 2-CH), 2.37 (s, 2H; 5-CH₂), 1.80 (s, 3H; OCH₃), 1.58 (s, 3H; OCH₃). IR (KBr) cm^{-1} : 3180, 1682, 1227, 1115, 784, 747, 722, 639. EI-MS *m/z*: 431.42 (Calcd for: 431.50). *Anal.* Calcd for $C_{24}H_{21}N_3O_3S$: C, 66.80; H, 4.90; N, 9.74. Found: C, 66.76; H, 4.83; N, 9.72.

Compound T₈: Yield = 80%, mp 198-200 °C, *R_f* = 0.48, 1H -NMR ($CDCl_3$) δ : 8.02 (m, 4H; B_Z), 7.63 (m, 4H; Ph), 6.90-7.44 (m, 4H; Ph), 4.15 (d, *J* = 2.2 Hz, 1H; 2-CH), 2.49 (s, 2H; 5-CH₂), 2.05 (s, 3H; OCH₃), 1.92 (s, 3H; OCH₃), 3.05 (s, 3H; O-CH₃), 2.92 (s, 3H; O-CH₃). IR (KBr) cm^{-1} : 3177, 1682, 1229, 1115, 784, 720, 637. EI-MS *m/z*: 461.42 (Calcd for $C_{25}H_{23}N_3O_4S$: 461.52). *Anal.* Calcd for: C, 65.06; H, 5.02; N, 9.17. Found: C, 64.97; H, 4.98; N, 9.14.



- (A₁) R₁=R₂=R₃=H
(A₂) R₁=R₃=H, R₂=Cl
(A₃) R₁=R₃=H, R₂=OH
(A₄) R₁=R₃=H, R₂=CH₃
(A₅) R₁=R₃=H, R₂=N(CH₃)₂
(A₆) R₁=R₃=H, R₂=OCH₃
(A₇) R₃=H, R₁=R₂=OCH₃
(A₈) R₁=R₂=R₃=OCH₃

- (T₁) R₁=R₂=R₃=H
(T₂) R₁=R₃=H, R₂=Cl
(T₃) R₁=R₃=H, R₂=OH
(T₄) R₁=R₃=H, R₂=CH₃
(T₅) R₁=R₃=H, R₂=N(CH₃)₂
(T₆) R₁=R₃=H, R₂=OCH₃
(T₇) R₃=H, R₁=R₂=OCH₃
(T₈) R₁=R₂=R₃=OCH₃

Scheme 1: Synthetic scheme of 2-[4-(azetidin-2-one)-3-Chloro-4-phenyl]-1H-Phenyl benzimidazoles and 2-(thiazolidin-4-one) Phenyl]-1H-Phenylbenzimidazoles

PHARMACOLOGICAL EVALUATION

Antimicrobial activity

The antibacterial activity^[15] of the synthesized compounds was tested against gram (+) bacteria (*Bacillus cereus* ATCC11778, *Micrococcus luteus* ATCC8341, *Staphylococcus aureus* ATCC9144 and *Salmonella epidermidis* ATCC155) and gram (-) bacteria (*Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* ATCC29665) using nutrient agar medium and fungi (*Aspergillus niger* ATCC9029 and *Candida albicans* ATCC2091) using sabourand dextrose agar medium.

Paper disc diffusion method

The sterilized (autoclaved at 120°C for 30 min), liquefied medium (40-50°C) was inoculated (1 ml/100 ml of medium) with the suspension of the microorganism (matched to McFarland barium sulphate standard) and poured into the petridish to give a depth of 3-4mm. The paper discs impregnated with the test compounds (50 µg/ml, dimethyl sulphoxide as solvent) were placed on the solidified medium. The plates were refrigerated (pre-incubated) for two hours at 4°C and then incubated at 37°C for 24 h and 48 h for antibacterial and antifungal activity respectively at the end of which the zone of inhibition was given in Table 1. Cefaclor (30 µg/disc) and fluconazole (100 µg/disc) were used as standards.

Minimum inhibitory concentration

The minimum inhibitory concentration^[16] (MIC) against the bacterial strains was determined by the test tube dilution technique using Mueller-Hinton nutrient broth. A series of glass tubes containing different concentrations of the synthesized compounds (in dimethyl sulphoxide) with the medium was inoculated with the required amount of inoculum to obtain a suspension of microorganism, which contains 10⁵ CFU/ml. One growth control tube was prepared without the addition of microorganism. The tubes were incubated at 37°C for 24 h. The minimum inhibitory concentration (MIC - µg/ml) was considered to be the lowest concentration that exhibited the same turbidity as the blank tube. The data are presented in Table 1.

Analgesic activity^[17-19]

The analgesic activity was determined by acetic acid induced writhing method-using wistar albino mice (n = 6) of either sex selected by random sampling technique (25-30 g) were used for the study. Diclofenac sodium at a dose level of 25 mg/kg served as standard drug for comparison. The negative control received solvent only (1% CMC). The test compounds at 200 mg/kg (suspended in 1% CMC) were administered orally by intragastric tube 30 min prior to intraperitoneal administration of the writhing agent (0.6 % v/v aqueous acetic acid-1ml/100g). The percent analgesic activity (PAA) was calculated by the following formula, where T₁ is the reaction time (s) before treatment, and T₂ is the reaction time (s) after treatment. The writhings produced in the animal were observed for 20 minutes and the percentage protection was calculated by following formula for analgesic activity. The results are given in Table 2.

$$\% \text{ Protection} = 100 - \left[\frac{\text{experimental}}{\text{control}} \times 100 \right]$$

Anti-inflammatory activity^[17-19]

The anti-inflammatory activity was evaluated by Carrageenan induced rat paw edema method. Albino rats of wistar strain weighing 150-200 g of either sex were divided into six animals in each group. 1 % CMC suspensions of the test compounds were administered intraperitoneally in a dose

Table 1: Antibacterial and Antifungal activity of the Title compounds(A₁₋₈ and T₁₋₈)

Compounds	Antibacterial activity						Antifungal activity	
	Zone of inhibition and Minimum Inhibitory Concentration (MIC)						Zone of inhibition (MIC)	
	BC	SA	ML	SE	EC	KP	AN	CA
A ₁	22 (75)	15 (75)	22 (75)	24 (65)	22 (80)	20 (80)	12 (85)	24 (65)
A ₂	25 (70)	17 (80)	22 (75)	25 (65)	24 (75)	26 (70)	08 (100)	20 (95)
A ₃	24 (70)	18 (80)	21 (75)	27 (50)	26 (80)	24 (75)	10 (90)	20 (85)
A ₄	22 (75)	15 (75)	20 (70)	23 (60)	18 (80)	21 (70)	14 (85)	24 (70)
A ₅	20 (60)	13 (75)	18 (75)	22 (60)	16 (80)	14 (80)	13 (85)	12 (100)
A ₆	24 (70)	15 (80)	19 (70)	20 (70)	15 (80)	15 (80)	15 (75)	22 (75)
A ₇	23 (75)	17 (85)	20 (75)	24 (65)	20 (70)	10 (80)	14 (90)	18 (80)
A ₈	24 (70)	16 (80)	21 (75)	26 (70)	19 (70)	16 (80)	16 (80)	17 (85)
T ₁	15 (50)	15 (75)	22 (65)	25 (70)	23 (65)	18 (80)	13 (85)	19 (80)
T ₂	20 (55)	17 (80)	20 (70)	18 (75)	25 (65)	20 (75)	12 (85)	24 (65)
T ₃	19 (60)	16 (75)	21 (70)	22 (75)	26 (65)	19 (75)	10 (90)	20 (65)
T ₄	17 (65)	15 (75)	22 (75)	20 (85)	22 (80)	16 (80)	13 (90)	22 (80)
T ₅	15 (50)	13 (80)	22 (65)	16 (80)	25 (75)	15 (80)	08 (100)	24 (65)
T ₆	16 (65)	12 (75)	18 (75)	18 (80)	20 (80)	14 (90)	12 (85)	24 (65)
T ₇	18 (70)	14 (75)	19 (85)	16 (80)	20 (75)	15 (80)	10 (85)	20 (90)
T ₈	19 (75)	11 (70)	20 (80)	20 (70)	18 (80)	14 (85)	11 (90)	20 (90)
Cefaclor	27	25	23	34	29	27	-	-
Fluconazole	-	-	-	-	-	-	18	23

Zone of Inhibition in mm & MIC in µg/ml

Significance levels: *p < 0.001 compared to control

Bacillus cereus (BC), *Staphylococcus aureus* (SA), *Micrococcus luteus* (ML), *Salmonella epidermidis* (SE), *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), *Aspergillus niger* (AN) and *Candida albicans* (CA).

Table 2: Analgesic activity and Anti-inflammatory activity of the Title compounds(1-16)

Compounds	Analgesic activity		Anti-inflammatory activity	
	Mean Writhings ± SEM	% Protection	Paw value Mean ± SEM	% Decrease in paw value
A ₁	17.33 ± 0.95*	54.99	0.28 ± 0.02*	65.0
A ₂	22.83 ± 1.08	40.70	0.32 ± 0.03*	60.0
A ₃	15.66 ± 0.8*	59.32	0.26 ± 0.02*	67.5
A ₄	19.3 ± 0.97*	49.87	0.25 ± 0.03*	68.75
A ₅	21.5 ± 1.9	44.16	0.28 ± 0.03*	65.0
A ₆	23.3 ± 1.4	39.48	0.31 ± 0.03*	61.25
A ₇	23.85 ± 1.5	38.05	0.27 ± 0.03*	66.25
A ₈	24.6 ± 1.8	36.10	0.35 ± 0.05	56.25
T ₁	10.3 ± 0.7*	73.25	0.22 ± 0.05*	72.5
T ₂	14.33 ± 0.85*	62.78	0.26 ± 0.05*	67.5
T ₃	8.2 ± 0.8*	78.70	0.21 ± 0.02*	73.75
T ₄	12.66 ± 1.2*	67.12	0.28 ± 0.02*	65.0
T ₅	13.85 ± 1.6*	64.03	0.30 ± 0.02*	62.5
T ₆	10.66 ± 1.1*	72.31	0.28 ± 0.02*	65.0
T ₇	9.5 ± 0.9*	75.32	0.30 ± 0.03*	62.5
T ₈	8.8 ± 0.74*	77.14	0.28 ± 0.02*	65.0
Control	38.5 ± 0.56	-----	0.80 ± 0.003	---
Diclofenac	7.5 ± 0.6*	80.52	0.30 ± 0.02*	62.50

Significance levels: *p < 0.001 compared to control

of 200 mg/kg. The control group was given only 1 % CMC suspensions. One group was administered with diclofenac sodium as standard drug, intraperitoneally in a dose of 25 mg/kg. After 30 min of the administration of test compounds paw edema was induced in albino rats injected carrageenin (0.1ml of 1.0 % solution in 0.9 % saline) into subplantar region of the left hind paw. After 4 h the increase in rat paw volume was recorded. The anti-inflammatory activity was measured in terms of percentage inhibition of edema of each group was calculated against control group. The given value is given in Table 2.

$$\% \text{ Reduction of edema} = \frac{[(\text{control-test})/\text{control}]}{1} \times 100$$

RESULTS AND DISCUSSION

2-(4-aminophenyl)-benzimidazole^[11] was treated with different substituted aromatic aldehydes to produce Schiff base.^[12] The Schiff bases were subjected to addition reactions with chloroacetyl chloride in the presence of triethylamine and mercaptoacetic acid in the presence of 1, 4-dioxane-anhydrous zinc chloride to produce 2-azetidinone derivatives and 4-thiazolidinone derivatives respectively.^[13-14] The chemical structures of the synthesized compounds

were confirmed by means of IR, ¹H-NMR, mass spectral and elemental analysis. The synthesized compounds were screened for antibacterial (*Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella epidermidis*), antifungal (*Aspergillus niger* and *Candida albicans*), analgesic activity by writhing reflex method and anti-inflammatory activity by carrageenin induced paw edema method. The synthesized compounds showed good antibacterial and antifungal activity against all tested organisms by disc diffusion method. The MIC of the compounds A₁₋₈ and T₁₋₈ for *S. aureus* (70-85 µg/ml), *B. cereus* (50-75 µg/ml), *E. coli* (65-80 µg/ml) *M. luteus*, (65-85 µg/ml) *K. pneumoniae*, (70-90 µg/ml) and *S. epidermidis* (60-85 µg/ml), antifungal *A. niger* (75-100 µg/ml) and *C. albicans* (65-100 µg/ml)). All the synthesized compounds showed significant analgesic activity by writhing reflex method and anti-inflammatory activity by carrageenin induced paw edema method. The thiazolidinone derivatives have good analgesic and anti-inflammatory activity in this prominent activity produced by 2-[2-(thiazolidin-4-one) Phenyl]-1H-Phenylbenzimidazole T₁ and 2-[2-(thiazolidin-4-

one) 4-hydroxyPhenyl]-1*H*-Phenylbenzimidazole **T₃** at the dose of 200 mg/kg was found to equivalent to diclofenac (25 mg/kg).

REFERENCES

1. Singh GS, Mmolotsi BJ. Synthesis of 2-azetidinones from 2-diazo-1, 2-diaryl ethanones and N-(2-thienylidene) imines as possible antimicrobial agents. *Il Farmaco*. 2005; 60(9): 727-730.
2. Rahman VPM, Mukhtar S, Ansari WH, Lemiere G. Synthesis, stereochemistry and biological activity of some novel long alkyl chain substituted thiazolidin-4-ones and thiazan-4-one from 10-undecenoic acid hydrazide. *Eur J Med Chem*. 2005; 40: 173-184.
3. Kucukguzel SG, Oruc EE, Rollas S, Sahin F, Ozbek A. Synthesis, characterization and biological activity of novel 4-thiazolidinones, 1,3,4-oxadiazoles and some related compounds. *Eur J Med Chem*. 2002; 37: 197-206.
4. Rawal RK, Prabhakar YS, Katti SB, De Clercq E. 2-(Aryl)-3-furan-2-yl-methyl-thiazolidin-4-ones as selective HIV-RT inhibitors. *Bioorg Med Chem* 2005; 13: 6771-6776.
5. Vigorita MG, Ottana R, Monforte F, Maccari R, Trovato A, Monforte MT, Taviano MF. Synthesis and anti-inflammatory, analgesic activity of 3, 3'-(1-2-Ethanedyl)-bis [2-aryl-4-thiazolidinone] chiral compounds part 10. *Bioorg Med Chem Lett*. 2001; 11: 2791-2794.
6. Goel B, Ram T, Tyagi R, Bansal E, Kumar A, Mukherjee D, Sinha JN. 2-Substituted-3-(4-bromo-2-carboxyphenyl)-5-methyl-4-thiazolidinones as potential anti-inflammatory agents. *Eur J Med Chem*. 1999; 34: 265-269.
7. Guven OO, Erdogan T, Goker H, Yildiz S. Synthesis and antimicrobial activity of some novel phenyl and benzimidazole substituted benzyl ethers. *Bioorg Med Chem Lett*. 2007; 17(8): 2233-6.
8. Mohamed BG, Abdel-Alim AA, Hussein MA. Synthesis of 1-acyl-2-alkylthio-1, 2, 4-triazolobenzimidazoles with antifungal, anti-inflammatory and analgesic effects. *Acta Pharm*. 2006; 56(1): 31-48.
9. Starcevic K, Kralj M, Ester K, Sabol I, Grce M, Pavelic K, Karminski-Zamola G. Synthesis, antiviral and antitumor activity of 2-substituted-5-amidino-benzimidazoles. *Bioorg Med chem*. 2007; 15(13): 4419-26.
10. Ayhan-Kilcigil G, Kus C, Ozdamar ED, Can-Eke B, Iscan M. Synthesis and antioxidant capacities of some new benzimidazole derivatives. *Arch Pharm*. 2007; 340(11): 607-611.
11. Mavrova ATs, Anichina KK, Vuchev DI, Tsenov JA, Denkova PS, Kondeva MS, Micheva MK. Antihelminthic activity of some newly synthesized 5(6) - un)substituted -1*H*-benzimidazol-2-ylthioacetyl-piperazine derivatives. *Eur J Med Chem*. 2006; 41(12): 1412-20.
12. Chua MS, Shi DF, Wrigley S, Bradshaw TD, Hutchinson I, Shaw PN, Barrett DA, Stanley LA, Stevens MFG. Antitumor benzothiazoles 7: Synthesis of 2(4-acylamino-phenyl)benzothiazoles and investigations into the role of acetylation in the antitumor activities of the parent amines. *J Med Chem*. 1999; 42(3): 381-392.
13. Conli M, Gulielmetli R, Metzger J. Synthesis, Physicochemical properties of schiff base in the benzothiazole series. *Bull Soc Chim Fr*. 1967; 8: 2834-2841.
14. Maurya MR, Kumar A, Ebel M, Rehder D, Synthesis, characterization, reactivity and catalytic potential of model vanadium (IV, V) complexes with benzimidazole-derived ONN donor ligands. *Inorg Chem*. 2006; 45(15): 5924-37.
15. Fahmy HH, El-Eraky W. Synthesis and evaluation of the analgesic and anti-inflammatory activities of o-substituted salicylamides. *Arch Pharm Res*. 2001; 24(3): 171-179.
16. More SV, Dongarkhadekar DV, Chavan RN, Jadhav WN, Bhusare SR, Pawar RP. Synthesis and antibacterial activity of new schiff bases, 4-thiazolidinones and 2-azetidinones. *J Indian Chem soc*. 2002; 79: 768-769.
17. Gillespie SH. "Medical Microbiology-Illustrated", Butterworth Heinemann Ltd., United Kingdom, 1994, pp. 234-247.
18. Hawkey PM, Lewis DA. "Medical Bacteriology-A practical approach", Oxford University Press, United Kingdom. 1994, pp. 181-194.
19. Turner RA. Peter Hebborn (Edt): "Screening Methods in Pharmacology", Vol. II, Academic Press, New York, 1971.