Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 11, 2015

Original Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-(2'/3'/4'/6'-SUBSTITUTED PHENYL)-1H-INDOLES

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Received: 20 Feb 2015 Revised and Accepted: 03 Oct 2015

ABSTRACT

Objective: Indole derivatives were reported to a wide range of biological activities. Thus it was our aim to synthesize a series of 2-(2'/3'/4'/6'-substituted phenyl) -1H-indoles using clayzic catalyst and screen for their *in vitro* anti-inflammatory, antioxidant and antimicrobial activities.

Methods: Various substituted acetophenones were reacted with phenylhydrazine in the presence of modified clayzic catalyst and obtained 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles in a one pot reaction. The cyclized compounds were characterized by FT-IR, NMR, UV-Vis and mass spectral analyses and screened for anti-inflammatory activity against cytokines tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) by measuring cytokine production by performing sandwich ELISA model, antioxidant activity by DPPH assay method and antimicrobial activity by well-diffusion method.

Results: An eco-friendly route with better yields for the synthesis of 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles in the presence of clayzic catalyst was achieved. The biological activity results suggested that compounds (2d, 2e and 2i) have excellent anti-inflammatory activity, compounds (2a-2d and 2j) possessing better antioxidant property and compounds (2b, 2i, 2k and 2m) have promising antibacterial and antifungal activities when compared to the standard drugs.

Conclusion: Synthesis of 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles was successfully achieved in the presence of clayzic catalyst. Compounds bearing amino, methyl, methoxy, hydroxyl and fluoro groups have shown better anti-inflammatory, antioxidant and antimicrobial activities when compared to the other compounds and 1H-indole.

Keywords: Fischer indole synthesis, 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles, Clayzic catalyst, *In vitro* anti-inflammatory activity, Antioxidant study, Antimicrobial activity.

INTRODUCTION

Generally, fused N-containing heterocyclic pharmacophore have a significant role in medicinal chemistry. Among them, indole nucleus occupies a unique position in heterocyclic chemistry due to its noticeable pharmacological properties and thus stands as an important scaffold in drug design. The most important anti-HIV drugs (Delavirdine, Atevirdine), anti-cancer drugs (Vincristine, Vinblastine) and the successful anti-inflammatory drug Indomethacin led to the exploration of various substituted indoles in order to develop better bioactive indoles. Furthermore, various 2-substituted indoles are well known for their widespread biological properties such as anti-inflammatory [1]. GABAA receptor antagonists [2], antioxidant [3], antibacterial [4], antifungal [5] and COX-inhibitors [6]. 2-Phenylindole-3-carbaldehyde was reported as an effective scaffold against breast cancer cell lines by inhibiting the polymerization of tubulin to functional microtubules [7]. These results prompted us to study the biological activities of various substituted indoles in more detail.

Inflammation is the body's response to the harmful stimuli, damaged cells and pathogens to begin the healing process. Chronic inflammation can lead to several diseases such as cancers, rheumatoid arthritis, atherosclerosis, periodontitis, and hay fever [8]. Nonsteroidal anti-inflammatory drugs (NSAIDs) can inhibit COX-2 enzyme and thereby inhibiting proliferation and inducing apoptosis of many malignant tumors [9, 10]. Studies have also demonstrated that NSAIDs can be effective in chemoprevention and treatment of many tumoral and cancer diseases [11, 12]. They also stand as an uncontrollable problem in the form of allergies and gastrointestinal risks. Thus, there is a need to develop new NSAIDs in order to avoid the side effects, to improve safety and tolerability. Antibiotic resistance is one of the serious problems in contemporary medicine and multidrug resistant microbes are thus becoming a serious threat to human life. Literature revealed that bacterial infection is strongly related to inflammation [13], the development of antibacterial agents having anti-inflammatory property is necessary to combat against these threats. In addition, antioxidants protect cells from reactive oxygen species, which lead to cancer, aging, atherosclerosis, ischemic injury, inflammation, and neurodegenerative diseases [14]. Hence, it was planned to synthesize the targets possessing various biological activities to inhibit the risks.

Although various catalytic systems for Fischer indole synthesis have been well studied in the literature, there is still continuing an effort to find the promoters to enhance the reaction rate under mild reaction conditions. The reported methods are time consuming, needs strong acid, high temperature and less purity of the desired products. The use of the moderate-strength Lewis acid, zinc chloride catalyst results in less waste and easy separation by water wash, it is widely used for Fischer indole cyclization. Nowadays, the use of reagents or catalysts on solid supports has experienced remarkable attention. Zinc chloride exchanged clay known as "clayzic" has been efficiently used as an environmentally friendly catalyst "Envirocat" in various Lewis acid catalyzed reactions even at room temperature [15-17]. Based on these considerations, we reported here the clayzic catalyzed synthesis of 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles and their *in vitro* anti-inflammatory, antioxidant, antibacterial and antifungal activities.

MATERIALS AND METHODS

Chemicals

All acetophenones and montmorillonite K-10 clay catalyst were purchased from Sigma-Aldrich Chemical & Co., and used without purification. The solvent methanol purchased from Thomas Baker was used. Phenylhydrazine hydrochloride and zinc chloride used were purchased from SD fine chemicals limited. Nutrient broth for bacterial and fungal studies was purchased from Hi-Media.

Instrumentation and analytical procedures

The melting point of all compounds was determined by open capillary melting point apparatus and uncorrected. FT-IR spectra were recorded in the Shimadzu IR Affinity-1 CE model with resolution 4 using KBr technique. UV spectra were recorded in a Jasco UV-Visible NIR spectrometer of model V670. The XRD patters were recorded in Bruker D8 Advance with CuKα radiation (Kαλ = 1.5406Å). The percentage conversion and molecular weight of the products were done by GC-MS chromatogram (Perkin Elmer system of GC model Clarus 680 and MS model Clarus 600 (EI)) using helium as a carrier gas. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 400MHz FT-NMR uses $CDCl_3$/DMSO-d_6 as a solvent and tetramethylsilane as internal standard. Chemical shifts are expressed in PPM.

Preparation of the clayzic catalyst

The catalyst was prepared by impregnating 1g K-10 clay (surface: 220-270 m^2/g; bulk density: 300-370 g/l) with 1g of powdered anhydrous zinc chloride in tetrahydrofuran (THF). The clayzic suspension was centrifuged, dried at 80^{o}C for 2h and grounded into a fine powder. Then, the catalyst was dried and stored in a moisture-free environment. Before using for the reaction, the catalyst was heated to 100^{o}C to remove the absorbed moisture. The XRD pattern in the fig. **1** clearly confirms the presence of the zinc on the active sites of the K-10 clay catalyst.

General procedure for the synthesis of 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles (2a-m)

Acetophenone (1a) (0.5 mmol) and phenylhydrazine (0.5 mmol) were dissolved in 10 ml methanol. The mixture was then warmed on the water bath and glacial acetic acid was added till the solution became clear. Clayzic catalyst (200 mg) was added to the above reaction mixture and refluxed. The reaction was monitored by TLC using petroleum ether and ethyl acetate (4:1). Upon completion of the reaction in 20 min, the reaction mixture was cooled to room temperature, filtered and poured the filtrate into the crushed ice. The solid K-10 clay catalyst that remained in the filter paper was thoroughly washed with a brine solution, dried and reused.

The product formed was then extracted with dichloromethane and roto-evaporated to get the crude product (2a). The crude product was purified by column chromatography using petroleum ether and ethyl acetate (90:10), yield (92%). The other substituted acetophenones (1b-m) were also subjected to the same procedure by utilizing 200-400 mg of the modified clayzic catalyst and obtained the cyclized compounds (2b-m) of about 60-90% yield. We observed better yield of the desired products in less reaction time when compared to the reported methods.

Characterization

2-Phenylindole (2a)

White solid; Yield: 92%; Melting point (^{o}C): 192 (Lit.: 184-185 [20]); IR(KBr) V_{max} (cm^{-1}): 3442.87 (-NH); ^{1}H NMR (400MHz, $CDCl_3$)δ: 6.835 (s, 1H$_{Arom}$), 7.106-7.181 (dd, 4H$_{Arom}$), 7.200 (t, 1H$_{Arom}$), 7.312-7.683 (m, 4H$_{Arom}$) 8.348 (s, 1H,-NH); ^{13}C NMR (100MHz, $CDCl_3$)δ: 100.01, 110.89, 120.29, 120.69, 122.38, 125.17, 127.74, 129.05, 129.28, 136.82, 137.89; GC-MS: 115, 165, 193.2511 (100%) (M^+); UV/vis λmax (MeOH): 235, 310 nm.

2-(4'-Aminophenyl) indole (2b)

Brown solid; Yield: 92%; m. p. (^{o}C): 207 (Lit: 201-202 [21]); IR(KBr) V_{max} (cm^{-1}): 3527.80 (-NH); 3257.77(-NH2); ^{1}H NMR (400MHz, DMSO)δ: 5.286 (s, 2H,-NH2), 6.583-7.536 (d, 4H$_{Arom}$), 6.728 (s, 1H$_{Arom}$), 7.202 (s, 4H$_{Arom}$), 8.946 (s, 1H,-NH); ^{13}C NMR (100MHz, DMSO)δ: 112.48, 113.42, 118.03, 126.21, 128.75, 130.53, 141.92, 146.62; GC-MS: 117, 152, 192, 208.2850 (100%) (M^+); UV/vis λmax (MeOH): 210, 325 nm.

2-(4'-Chlorophenyl) indole (2c)

White solid; Yield: 86%; m. p. (^{o}C): 193 (Lit.: 193-195 [20]); IR(KBr) V_{max} (cm^{-1}): 3442.94 (-NH); ^{1}H NMR (400MHz, $CDCl_3$)δ: 6.814 (s, 1H$_{Arom}$), 7.117-7.912 (m, 8H$_{Arom}$), 8.287 (s, 1H,-NH); ^{13}C NMR (100MHz, $CDCl_3$)δ: 100.62, 111.08, 120.61, 120.90, 122.83, 126.46, 129.31, 129.37, 131.04, 133.59, 136.82, 137.05; GC-MS: 114, 165, 191, 227.1465 (100%) (M^+), 229; UV/vis λmax (MeOH): 245, 315 nm.

2-(4'-Methylphenyl) indole (2d)

White solid; Yield: 70%; m. p. (^{o}C): 219 (Lit.: 210-212 [20]); IR(KBr) V_{max} (cm^{-1}): 3433.29 (-NH); ^{1}H NMR (400MHz, $CDCl_3$)δ: 2.208(s, 3H,-CH3), 6.844-6.880 (t, 1H$_{Arom}$), 7.165-7.183 (d, 4H$_{Arom}$), 7.238-7.293 (t, 1H$_{Arom}$),7.289 (s, 1H$_{Arom}$), 7.673-7.693(d, 2H$_{Arom}$); ^{13}C NMR (100MHz, $CDCl_3$)δ: 26.85, 122.39, 122.99, 126.43, 126.88, 128.44, 129.07, 129.24, 130.39, 131.13, 145.91, 152.35; GC-MS: 115, 178, 191, 207.1427 (100%) (M^+); UV/vis λmax (MeOH): 205, 240, 345 nm.

2-(4'-Methoxyphenyl) indole (2e)

White flakes; Yield: 65%; m. p. (^{o}C): 226 (Lit.: 226-227 [22]); IR(KBr) V_{max} (cm^{-1}): 3429.43(-NH); ^{1}H NMR (400MHz, CDCl3)δ: 3.833(s, 3H,-OCH3), 6.842-6.904(t, 2H$_{Arom}$), 6.921 (s, 1H$_{Arom}$), 7.157-7.293 (d, 4H$_{Arom}$),7.729-7.750(d, 2H$_{Arom}$); ^{13}C NMR (100MHz, CDCl3)δ: 55.35, 113.17, 113.70, 113.74, 119.97, 126.88, 129.24, 130.62, 145.46, 159.67; GC-MS: 117, 131, 207, 224.2504 (M^+); UV/vis λmax (MeOH): 240, 295, 325 nm.

2-(3'-Nitrophenyl) indole (2f)

Pale yellow crystals; Yield: 85%; m. p. (^{o}C): 176 (Lit.: 172 [23]); IR(KBr) V_{max} (cm^{-1}): 3425.58 (-NH); ^{1}H NMR (400MHz, CDCl3)δ: 7.031 (s, 1H$_{Arom}$), 7.150-7.295(t, 2H$_{Arom}$), 7.434-8.320 (d, 6H$_{Arom}$), 8.444(s, 1H,-NH); ^{13}C NMR (100MHz, CDCl3)δ: 103.62, 111.40, 121.13, 121.52, 124.14, 124.73, 125.30, 129.09, 138.55, 196.45; LC-MS: 116, 238.2014 (100%) (M^+); UV/vis λmax (MeOH): 235, 265, 385 nm.

2-(4'-Nitrophenyl) indole (2g)

Yellow crystals; Yield: 90%; m. p. (^{o}C): 178-180 (Lit.: 188-190 [20]); IR(KBr) V_{max} (cm^{-1}): 3429.43 (-NH); ^{1}H NMR (400MHz, CDCl3)δ: 7.032(s, 1H$_{Arom}$), 7.150-7.296 (t, 2H$_{Arom}$), 7.435-8.322 (d, 6H$_{Arom}$), 8.453(s, 1H,-NH); ^{13}C NMR (100MHz, CDCl3)δ: 103.48, 111.27, 120.99, 121.39, 123.90, 124.00, 124.59, 125.17, 128.96, 196.35; LC-MS: 117, 174, 238.10 (100%) (M^+); UV/vis λmax (MeOH): 230, 265, 390 nm.

2-(2', 6'-Dimethylphenyl) indole (2h)

White solid; Yield: 61%; m. p. (^{o}C): 184-170; IR(KBr) V_{max} (cm^{-1}): 3414.00(-NH); ^{1}H NMR (400MHz, DMSO)δ: 2.434-2.497(s, 6H,-CH3), 6.796-6.816 (d, 2H$_{Arom}$), 6.927-6.971 (t, 1H$_{Arom}$), 7.069 (s, 1H$_{Arom}$), 7.159-7.271 (t, 2H$_{Arom}$), 7.344-7.379(d, 2H$_{Arom}$), 7.512 (s, 1H,-NH); LC-MS: 117, 191, 206, 221.80 (100) (M^+); UV/vis λmax (MeOH): 225, 275, 305 nm.

2-(3', 4'-Dimethoxyphenyl) indole (2i)

White flakes; Yield: 62%; m. p. (^{o}C): 178-192 (Lit.: 190 [24]); IR(KBr) V_{max} (cm^{-1}): 3415.93(-NH); ^{1}H NMR (400MHz, CDCl3)δ: 3.871-3.903(s, 6H,-OCH3), 6.157-6.293 (d, 2H$_{Arom}$), 6.521(s, 1H$_{Arom}$), 7.043(s, 1H$_{Arom}$), 7.138-7.182(t, 2H$_{Arom}$),7.729-7.750(d, 2H$_{Arom}$); GC-MS: 149(100%), 253.2663 (M^+); UV/vis λmax (MeOH): 210, 247, 277, 315 nm.

2-(2'-Hydroxyphenyl) indole (2j)

Greenish yellow crystals; Yield: 82%; m. p. (^{o}C): 160-162 (Lit.: 184-185 [26]); ^{1}H NMR (400MHz, CDCl3)δ: 5.585 (s,-OH), 6.868 (s, 1H$_{Arom}$), 6.889-6.923(d, 2H$_{Arom}$), 7.007-7.030(dd, 1H$_{Arom}$), 7.169-7.206(t, 1H$_{Arom}$), 7.348-7.352(d, 1H$_{Arom}$), 7.371-7.410(t, 2H$_{Arom}$),7.616-7.640(dd, 1H$_{Arom}$), 14.651(s,-NH); ^{13}C NMR (100MHz, CDCl3)δ: 115.12, 118.14, 118.28, 119.77, 121.31, 124.82, 128.93, 129.09, 133.07, 136.50, 147.03, 162.01, 171.18; GC-MS: 120, 167, 196 (100%), 211 (M+2); UV/vis λmax (MeOH): 221, 257, 321 nm.

2-(4'-Fluorophenyl) indole (2k)

Off white solid; Yield: 78%; m. p. (^{o}C): 188-190 (Lit.: 188 [25]); ^{1}H NMR (400MHz, DMSO)δ: 6.710-6.729 (d, 4H$_{Arom}$), 7.123-7.141 (d, 4H$_{Arom}$), 7.992(s, 1H$_{Arom}$); GC-MS: 151, 170, 199 (100%), 213.2732 (M+2); UV/vis λmax (MeOH): 203, 232, 286 nm.

2-(2'-Bromophenyl) indole (2l)

Pale yellow solid; Yield: 62%; m. p. (°C): 70-72 (75-77 [27]); ^{1}H NMR (400MHz, $CDCl_3$)δ: 6.93 (s, $1H_{Arom}$), 7.415-7.891 (m, $8H_{Arom}$), 11.37 (s, 1H,-NH); GC-MS: 117, 195, 272 (100%), 274.1039 (98%) (M+2); UV/vis λmax (MeOH): 222, 281 nm.

2-(4'-Bromophenyl) indole (2m)

Off white solid; yield: 80%; m. p. (°C): 208-210; ^{1}H NMR (400MHz, $CDCl_3$)δ: 6.834 (s, $1H_{Arom}$), 7.217-7.992 (m, $8H_{Arom}$), 8.87 (s, 1H,-NH); ^{13}C NMR (100MHz, $CDCl_3$)δ: 100.06, 111.18, 120.42, 120.78, 122.18, 126.26, 129.11, 129.30, 131.64, 133.69; GC-MS: 116, 178, 272 (100%) (M^+), 274.2528 (98%) (M+2); UV/vis λmax (MeOH): 221, 285, 318 nm.

***In vitro* anti-inflammatory assay**

Inflammation IL-6 and TNF-α screening

The human monocytic cell line THP-1 (ATCC, Manassas, VA) was maintained in RPMI-1640 (Bioconcept) supplemented with 10 % FBS (GIBCO). Prior to LPS-stimulation, 25,000 cells per well were cultured for 24 h in the presence of 10 ng/ml of phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) to enhance their response to activation stimuli. These cells were then stimulated with 1 µg/ml LPS (Sigma-Aldrich) for 24 h in the presence or absence of 10 µg/ml extract at 0.5% DMSO concentration. At the end of 24 h, the cell supernatant was used for cytokine measurements, and the cells used for determining the toxic effects of the compounds by exposure to CCK-8 reagent from Do Jindo. For all experiments, the THP-1 monocytes were used only up to passage # 25 and were utilized within 2 mo post revival of a new vial.

Cytokine determination

The monocytes were maintained in RPMI-1640 supplemented with 10% FBS, and cytokine expression in the presence or absence of the compound was determined. The levels of cytokines generated were assessed by ELISA from BD Biosciences having a sensitivity threshold of 4.6 pg/ml for IL-6 and 7.8 pg/ml for TNF-α. Simultaneously, assays were performed to determine the effect of the compound on cellular viability using CCK-8, a dehydrogenase activity measurement kit.

***In vitro* antimicrobial assay**

All the synthesized 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles were screened for their *in vitro* antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus aureus* and antifungal activity against *Candida albican, Aspergillus fumigates and Aspergillus niger* according to agar well diffusion method [18]. The inoculated suspensions of organisms in sterile distilled water were uniformly distributed to a density of 5 mm with a glass spreader onto the sterilized petri dishes (25 ml) and allowed to solidify for at least 3 min but no longer than 15 min before making wells. A hollow tube of 5 mm diameter was heated and pressed on above the inoculated agar plates and was removed immediately. Similarly, five wells were made on each plate. Sample stock solutions were prepared to about 1 mg/ml by using DMSO as a solvent and which were serially added (50, 100, 150 and 200 µl) to each well with the help of a micropipette. The plates were incubated for 18-24 h at 37°C in an incubator. Amoxicillin and Clotrimazole were used as the positive control and DMSO as blank. The plates were read only if the lawn of growth was confluent or nearly confluent. The diameter of the inhibition zone was measured to the nearest whole millimeter by holding the measuring device. The obtained results were represented in table 1 revealed that all the compounds possessing moderate to good antibacterial and antifungal activities.

Minimum inhibitory concentration (MIC) of the compounds 2b, 2i and 2k against *E. coli & Pseudomonas aeruginosa* (Gram negative Bacteria) and compounds 2i and 2m against *Candida albicans* and *Aspergillus niger* were determined by the Broth Micro dilution method. The stock solutions of the compounds and the drugs were prepared by using DMSO, which had no effect on the organisms in the preliminary studies. Various concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.56 µg/ml) of the compounds and the drugs were prepared. The lowest concentration that resists the micro-organisms growth was noted as the MIC and represented the results in table 2.

DPPH radical scavenging assay

The radical scavenging activity of synthesized compounds against 2, 2-diphenyl-2-picyrl hydrazyl hydrate (DPPH) was determined by using Brand-Williams *et al.* (1995) method [19]. Ascorbic acid was used as a standard. The reaction mixture contains 0.4 ml of 1 mmol freshly prepared DPPH, different volume (80, 160, 240, 320 and 400 µl) of 1 mg/ml solution of the compounds and the required volume of ethanol to make the whole mixture to 4 ml. A blank was prepared without the addition of the samples. After additions, the reaction mixtures were kept in the dark at room temperature for 30 min. The change in color (from violet to yellow) was observed, and their absorbance was measured at 517 nm by using UV-Vis spectrophotometer. Lower the absorbance of the mixture indicates the higher radical scavenging activity. The experiment was done in triplicate to find the mean and standard deviation. % Radical scavenging activity was calculated by using the following equation:

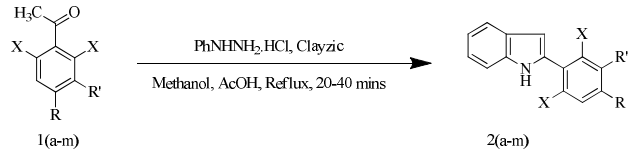
$$\% \text{ Inhibition } = \left(\frac{(AB - AS)}{AB}\right) X\ 100$$

Where, AB–Absorbance of blank sample (t = 0 min); AS–Absorbance of test samples (t = 30 min).

RESULTS AND DISCUSSION

Chemistry

As we aim to achieve the targets in mild reaction conditions with higher yields and in less reaction time, the reaction has been carried out thermally in the presence of a modified clayzic catalyst as shown in Scheme 1. The reaction was completed much earlier and also the yields were better when compared to the use of other catalysts. It is expected that the Lewis acidity of the cations on the edge sites on the K-10 clay catalyst was accelerated by the addition of the zinc chloride. It has also been observed that the rate of the reaction depends on the nature of the substituents in the acetophenones and the solvent used.



1(a-m) 2(a-m)

R = H, NH_2, Cl, CH_3, OCH_3, F, Br, NO_2; R' = H, NO_2, OCH_3; X = H, CH_3, OH, Br

Scheme 1: Clayzic catalyzed Fischer indole synthesis from various substituted acetophenones

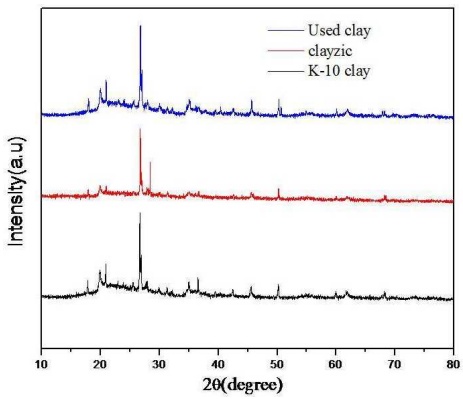


Fig. 1: XRD patterns of Clay, Clayzic and used clay catalysts

The chloro, methyl, nitro, bromo and fluoro substituted acetophenones underwent cyclization in very short time comparatively and the polar solvent methanol favored the reaction.

The zinc salt was easily washed off with water and the montmorillonite K-10 clay catalyst was finally recovered from the reaction mixture by filtration, washed with brine solution, dried and reused. The characteristics of the clay catalyst, depending on its active sites were also evidenced by their XRD results in fig. 1. The activity of the clay catalyst was effective up to 3-5 times. The successful results prompted us to go for one pot synthesis of the series of 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles in the methanol medium by thermal method.

Biological screening

Antimicrobial study

Antibacterial and antifungal screening results of the compounds (2a-2m) revealed their slight to moderate activity against chosen bacterial and fungal strains ranged from 60% of the standards. All the compounds have shown better activity against *Pseudomonas aeruginosa* and low activity against *Aspergillus fumigates.* In addition, it was found that the activities of the substituted indoles were better than the unsubstituted 1H-indole. Indoles with 4'-aminophenyl, 2', 6'-dimethylphenyl, 4'-fluorophenyl and 2'-bromophenyl at its second position possessing better activity than the other substituted indoles.

Compounds (2b, 2i, 2k and 2m) were screened for MIC studies. Compounds (2i and 2k) were shown better antibacterial activity with MIC of 9.37µg/ml against *E. coli* and 9.37µg/ml against *Pseudomonas aeruginosa*. Compound (2i) showed better antifungal activity with MIC of 9.37µg/ml against *Candida albicans* and 9.37 µg/ml against *Aspergillus niger.* Antimicrobial and MIC results have been clearly tabulated in table 1 & table 2.

Anti-inflammatory study

The anti-inflammatory activity of the synthesized compounds at different concentration (10, 30, 50, 80, 100 µM) was evaluated and listed the results for 50 µM concentration along with their IC_{50} value in table 3. The anti-inflammatory results showed that compounds (2d, 2e and 2i) bearing 4'-methylphenyl, 4'-methoxyphenyl and 3', 4'-dimethoxyphenyl at the 2-position of the indole shown comparable inhibition with the standard. Also, the compound (2f) bearing nitro substituent at the *meta* position of the phenyl ring shown less inhibition compared to the same substituent at the *para* position as in the compound (2g).

The electron releasing substituent's on the phenyl ring of the compounds (2b and 2h) have shown less activity. No toxicity was observed upto 50 µM concentration of all the compounds as shown in table 3. Thus, the overall study implies that the 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles possessing significant anti-inflammatory activity along with antibacterial and antifungal activities.

Table 1: Antimicrobial results of 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles (2a-m)

Compounds	**Zone of inhibition (Mean+SD) in mm (--not tested)**													
Organisms	**Bacteria**								**Fungi**					
	Escherichia coli		***Pseudomonas aeruginosa***		***Staphylococcus aureus***		***Bacillus subtilis***		***Aspergillus niger***		***Aspergillus fumigates***		***Candida albicans***	
µl of stock sample (1 mg/ml)	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**
Indole	9.67+0.58	12.33+2.08	11.00+1.73	12.33+1.15	8.67+0.58	12.33+0.58	7.67+0.58	11.67+0.58	7.33+0.58	11.67+0.58	7.33+1.53	9.67+0.58	7.67+1.53	11.67+0.58
2a	6.67+1.53	10.67+1.15	6.67+0.58	11.67+0.58	8.33+0.58	11.67+0.58	7.33+0.58	10.67+0.58	9.67+1.15	12.33+1.15	7.33+1.53	10.33+1.15	9.67+2.08	12.67+0.58
2b	20.67+0.58	27.67+0.58	20.33+1.53	24.67+0.58	11.33+0.58	15.67+0.58	11.67+0.58	16.33+0.58	11.33+0.58	14.33+1.15	7.33+1.53	9.67+0.58	13.00+1.00	15.33+1.15
2c	10.33+0.58	13.67+0.58	10.67+0.58	14.67+0.58	8.331.15	12.67+0.58	9.67+0.58	12.67+1.53	9.67+0.58	12.33+1.15	7.33+1.53	10.33+1.15	10.00+1.73	12.67+0.58
2d	11.33+1.53	12.33+0.58	10.67+1.53	12.33+1.15	9.67+0.58	12.00+1.00	8.67+0.58	11.67+0.58	10.33+0.58	12.33+1.15	7.33+1.53	9.67+0.58	10.33+1.53	12.67+0.58
2e	10.67+0.58	14.67+0.58	11.33+0.58	13.67+0.58	7.33+0.58	9.67+0.58	7.67+0.58	10.33+0.58	9.33+0.58	12.33+1.15	7.33+1.53	10.33+1.15	9.67+1.15	12.33+1.15
2f	10.67+1.53	14.33+1.53	10.33+1.15	14.33+0.58	9.67+0.58	11.67+0.58	7.33+0.58	11.33+0.58	10.67+0.58	12.33+1.15	8.33+2.52	11.67+0.58	10.00+1.73	13.33+1.15
2g	9.67+1.53	12.33+2.08	10.67+0.58	14.33+0.58	9.33+0.58	12.67+0.58	9.67+0.58	11.33+0.58	8.67+0.58	12.33+1.15	7.33+1.53	9.67+0.58	9.33+1.53	12.33+1.15
2h	10.67+1.53	13.67+0.58	11.67+1.15	14.33+0.58	7.33+0.58	10.67+0.58	7.33+0.58	11.67+0.58	10.67+1.15	12.33+1.15	7.33+1.53	9.67+0.58	10.00+1.73	14.00+1.15
2i	24.67+0.58	31.33+1.53	22.33+1.53	26.67+0.58	11.33+0.58	16.33+0.58	11.33+0.58	16.67+0.58	14.67+0.58	20.00+1.15	12.00+2.00	15.33+1.15	16.33+1.53	19.33+1.15
2j	6.67+0.58	9.67+0.58	7.33+1.53	9.33+0.58	7.33+0.58	10.33+0.58	7.33+0.58	10.33+0.58	10.33+0.58	12.33+1.15	7.33+1.53	10.33+1.15	10.67+1.53	12.67+0.58
2k	21.67+0.58	28.00+1.00	26.67+1.53	34.67+0.58	10.33+1.53	12.67+0.58	10.33+0.58	12.33+1.15	12.67+0.58	15.33+1.15	9.33+1.53	12.33+1.15	12.67+1.15	15.33+1.15
2l	12.33+1.53	16.33+0.58	11.33+0.58	15.33+0.58	7.67+0.58	12.33+0.58	9.67+0.58	12.33+0.58	17.33+0.58	22.00+1.73	11.00+2.00	14.33+1.15	21.33+1.53	24.33+1.15
2m	9.67+1.53	12.67+0.58	11.33+1.53	14.33+0.58	7.67+0.58	11.33+0.58	7.33+1.53	11.33+0.58	7.67+0.58	11.67+0.58	7.33+1.53	10.33	9.33+1.53	12.33+1.15
Amoxicillin	41+0.58	-	42+0.58	-	41+0.58	-	41+1.15	-	-	-	-	-	-	-
Clotrimazole	-	-	-	-	-	-	-	-	40+0.58	-	40+0.58	-	44+0.58	-

Table 2: MIC results of compounds (2b, 2i, 2k and 2m) shown better zone of inhibition

Bacteria:	***Against escherichia coli***								***Against pseudomonas aeruginosa***							
Samples (µg/ml)	100	50	25	12.5	6.25	3.125	1.56	MIC	100	50	25	12.5	6.25	3.125	1.56	MIC
2b	S	S	S	R	R	R	R	18.7+3.15	S	S	S	R	R	R	R	18.7+3.15
2i	S	S	S	S	R	R	R	9.37+1.56	S	S	S	S	R	R	R	9.37+1.56
2k	S	S	S	S	R	R	R	9.37+1.56	S	S	S	S	R	R	R	9.37+1.56
Amoxicillin	S	S	S	S	S	S	R	2.34+0.39	S	S	S	S	S	S	R	2.34+0.39
Fungi:	*Against Candida albicans*								*Against Aspergillus niger*							
2i	S	S	S	S	R	R	R	9.37+1.56	S	S	S	S	R	R	R	9.37+1.56
2m	S	S	S	S	R	R	R	9.37+1.56	S	S	S	R	R	R	R	18.7+3.15
Clotrimazole	S	S	S	S	S	R	R	4.68+0.78	S	S	S	S	R	R	R	9.37+1.56

R-resistant; S-sensitive; MIC: Mean+SD in µg/ml

Table 3: Anti-inflammatory results of 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles (2a-m)

Compounds	**Anti-inflammatory results**					**% Toxicity (Concentration in µM)**
	Concentration in µM	**% inhibition (Mean+SD)**		**IC_{50} in µM**		
		TNF-α	**IL6**	**TNF-α**	**IL6**	
2a	50	48.76+1.26	84.97+0.65	50	30	0 (200)
2b	50	42.24+0.81	84.95+0.92	55	20	4 (200)
2c	50	49.38+1.26	86.25+1.04	50	20	11 (100)
2d	50	77.54+0.25	94.87+0.94	25	10	7 (130)
2e	50	70.39+0.48	92.54+0.17	30	10	20 (130)
2f	50	51.09+0.90	90.40+0.31	50	10	35 (80)
2g	50	11.65+1.45	92.28+0.95	120	20	5 (80)
2h	50	2.47+0.12	71.27+0.68	120	30	3 (100)
2i	50	68.33+1.03	97.31+0.87	30	10	4 (130)
2j	50	48.21+1.25	86.26+1.11	50	30	17 (80)
2k	50	52.01+0.28	79.57+0.17	50	30	12 (100)
2l	50	50.97+1.01	93.23+0.65	50	10	10 (80)
2m	50	49.70+0.72	63.17+0.22	50	30	12 (100)
Indole	50	47.13+0.85	59.48+0.70	50	30	0 (200)
Standard drug	10	60.08+0.28	63.65+0.21	<10	<10	0(200)

Antioxidant study

The DPPH radical scavenging method is extensively used to evaluate the antioxidant activity in shorter time duration. DPPH, which is a stable free radical, can accept hydrogen radical or an electron shows a strong absorption band at 517 nm. This assay measures the electron donor capability of the compounds.

The color of DPPH changes from violet to yellow on reduction by the compounds. The antioxidant results are tabulated in table 4. Results suggested that compounds (2a, 2c, 2d, 2k and 2m) possessing better radical scavenging activity (85, 88, 71, 86 and 76%) at 100 µg/ml and the remaining compounds shown moderate. The IC_{50} values of all the screened compounds have been calculated and tabulated.

Table 4: DPPH radical scavenging activity of compounds (2a-2m)

Compounds	**% Radical scavenging activity (Mean+SD)**					**(µg/ml)**
	Concentration (µg/ml)					
	20	**40**	**60**	**80**	**100**	**IC_{50}**
Ascorbic acid	52.30+0.01	88.27+0.04	90.99+0.03	94.22+0.08	100.09	<20
2a	9.18+0.03	32.40+0.04	56.21+0.08	82.40+0.11	85.27+0.13	55+0.06
2b	1.53+0.01	34.95+0.05	55.19+0.02	58.78+0.01	61.28+0.03	55+0.04
2c	25.17+0.03	31.72+0.11	56.38+0.05	58.67+0.03	88.20+0.13	55+0.08
2d	17.18+0.05	31.89+0.09	58.84+0.05	69.56+0.02	71.51+0.09	46+0.07
2e	10.03+0.03	17.18+0.05	23.81+0.02	55.19+0.03	68.11+0.08	77+0.03
2f	0.51+0.04	16.67+0.07	33.76+0.01	36.99+0.05	53.40+0.08	96+0.03
2g	5.10+0.01	26.87+0.11	34.44+0.04	51.28+0.04	56.63+0.10	79+0.08
2h	9.01+0.01	43.03+0.12	49.23+0.01	63.01+0.04	70.32+0.11	61+0.03
2i	7.99+0.04	18.88+0.01	22.28+0.04	48.91+0.03	49.89+0.12	100+0.03
2j	6.89+0.04	15.22+0.03	55.70+0.03	77.64+0.01	86.31+0.11	57+0.03
2k	7.91+0.02	11.82+0.02	25.43+0.11	37.33+0.03	43.37+0.09	>100
2l	1.45+0.01	11.05+0.06	30.19+0.05	33.42+0.02	52.47+0.10	98+0.03
2m	3.32+0.01	9.44+0.05	24.49+0.03	55.95+0.02	76.92+0.10	76+0.03

CONCLUSION

We have successfully achieved the synthesis of 2-(2'/3'/4'/6'-substituted phenyl)-1H-indoles in the presence of clayzic catalyst and screened their antimicrobial, anti-inflammatory and antioxidant activities. All compounds have shown moderate to good activity. Among them, 2-(4'-methylphenyl) indole (2d) showed better inhibitory activity against IL-6 (81%) when compared to the standard drug Dexamethasone (63%). All compounds were tested for toxicity and found safety equivalent to the standards. 2-(4'-Aminophenyl) indole (2b) and 2-(4'-fluorophenyl) indole (2k) were identified as good antibacterial agents against *Pseudomonas*

aeruginosa and *Escherichia coli* respectively, and compound (2i) was identified as a good antifungal agent against *Aspergillus niger* and *Candida albicans*. All the compounds were shown good to better antioxidant property. Thus, further pharmacological and phytochemical analyses are required to develop new bioactive molecules.

ACKNOWLEDGEMENT

We are very much thankful to VIT University, Vellore for their financial support, VIT-DST SIF, Vellore for NMR characterization and GC-MS analysis and our thanks are also to Dr. Dattatraya Desai and Dr. Mini Dhiman for their wholehearted help. We thank VIT-TBI for FT-IR and UV-Visible spectra.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Dhaneshwar SR, Chaturvedi SC, Christiana. Synthesis and anti-inflammatory activities of some n-acetyl-2-phenylindole mannich bases. Indian J Pharm Sci 1988;50:165-7.
2. Luis FJ, Pique M, Gonzalez M, Buira I, Mendez E, Terencio J, *et al*. Synthesis, pharmacology and molecular modeling of N-substituted 2-phenyl-indoles and benzimidazoles as potent GABAA agonists. Eur J Med Chem 2006;41:985-90.
3. Sibel S, Pinar B, Tulay C, Dogu N. Investigation of the *in vitro* antioxidant behavior of some 2-phenylindole derivatives: discussion on possible antioxidant mechanisms and comparison with melatonin. J Enzym Inhib Med Chem 2006;21:405-11.
4. Leboho TC, Michael JP, Van Otterlo WAL, Van Vuuren SF, De Koning CB. The synthesis of 2-and 3-aryl indoles and 1,3,4,5-tetrahydropyrano[4,3-b]indoles and their antibacterial and antifungal activity. Bioorg Med Chem Lett 2009;19:4948-51.
5. Sharma P, Ashok K, Vinita S, Siya U, Jitendra S. Synthesis of bioactive spiro-2-[3'-(2'-phenyl)-3H-indolyl]-1-aryl-3-phenyl aziridines and SAR studies on their antimicrobial behavior. Med Chem Res 2009;18:383-95.
6. Mitsuhiro A, Yayoi K, Tohru O, Takuma S, Tomonori N, Takuya A, *et al*. Design and synthesis of Indomethacin analogues that inhibit P-glycoprotein and/or multidrug resistant protein without COX inhibitory activity. J Med Chem 2012;55:8152-63.
7. Kaufmann D, Pojarova M, Vogel S, Liebl R, Gastpar R, Gross D, *et al*. Antimitotic activities of 2-phenylindole-3-carbaldehydes in human breast cancer cells. Bioorg Med Chem 2007;15:5122–36 (b) Gastpar R, Goldbrunner M, Marko D, Von Angere E. Methoxy-substituted 3-formyl-2-phenylindoles inhibit tubulin polymerization. J Med Chem 1998;41:4965–72.
8. http://www.medicalnewstoday.com/articles/248423.php. [Last accessed on 2015 Jan 10].
9. Goossens L, Pommery N, Henichart JP. CoX-2/5LoX dual acting anti-inflammatory drugs in cancer chemotherapy. Curr Top Med Chem 2007;7:283-96.
10. Liu W, Zhou J, Bensdorf K, Zhang H, Liu H, Wang Y, *et al*. Investigations on cytotoxicity and anti-inflammatory potency of licofelone derivatives. Eur J Med Chem 2011;46:907-13. (b) Zhou JP, Ding YW, Zhang HB, Xu L, Dai Y. Synthesis and anti-inflammatory activity of imidazo [1,2-a]pyrimidine derivatives. Chin Chem Lett 2008;19:669-72.
11. Pereg D, Lishner M. Nonsteroidal anti-inflammatory drugs for the prevention and treatment of cancer. J Int Med 2005;258:115-23.
12. Agrawal A, Fentiman IS. NSAIDs and breast cancer: a possible prevention and treatment strategy. Int J Clin Pract 2008;62:444-9.
13. Sy M, Kitazawa M, Medeiros R, Whitman L, Cheng D, Lane ET, *et al*. Inflammation induced by infection potentiates tau pathological features in transgenic mice. Am J Pathol 2011;178:2811–22.
14. Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radical Res 1999;31:261-72.
15. Clark JH, Cullen SR, Barlow SJ, Bastock TW. Environmentally friendly chemistry using supported reagent catalysts–Structure property relationships for clayzic. J Chem Soc Perkin Trans 1994;2:1117-30.
16. Varala R, Enugala R, Adapa SR. Zinc montmorillonite as a reusable heterogeneous catalyst for the synthesis of 2,3-dihydro-1H-1,5-benzodiazepine derivatives. ARKIVOC 2006;13:171-7.
17. Dhakshinamoorthy A, Kanagaraj K, Pitchumani K. Zn2+-K10-clay (clayzic) as an efficient water-tolerant, solid acid catalyst for the synthesis of benzimidazoles and quinoxalines at room temperature. Tetrahedron Lett 2011;52:69-73.
18. Barry AL. The antimicrobic susceptibility test: principles and practices (Lea & Febiger Publishers); 1976. p. 180.
19. Brand-williams W, Cuvelier ME, Berset C. Lebensmittel wissenschaft und technologie. J Impact Factor Inf 1995;28:25-30.
20. Dhakshinamoorthy A, Pitchumani K. Facile clay-induced fischer indole synthesis: A new approach to synthesis of 1,2,3,4-tetrahydrocarbazole and indoles. Appl Catal A 2005;292:305-11.
21. Afshin Zarghi, Azar Tahghighi, Zohreh Soleomani, Bahram Daraie, Orkideh Gorban Dadrass, Mehdi Hedayati. Design and synthesis of some 5-Substituted-2-(4-(azido or methylsulfonyl)phenyl)-1H-indole derivatives as selective cyclooxygenase (COX-2) Inhibitors. Sci Pharm 2008;76:361–76.
22. Lutz Ackermann, Alexander V. Ruthenium-catalyzed direct C-H bond arylations of heteroarenes. Org Lett 2011;13:3332-5.
23. Bansal RK, Sharma SK. Reaction of phenacyltriphenylarsonium bromide with aromatic primary amines: synthesis of 2-arylindoles and 2-arylbenzindoles through arsenic ylide. J Organomet Chem 1978;149:309-14.
24. Bruce J Malcolm. Heterocyclic compounds of nitrogen. Part III. The synthesis of some 2-indolylbenzoquinones. J Chem Soc 1960;360-65.
25. Li Pinhua, Lei Wang, Feng You. Gold(I)Iodide catalyzed sonogashira reactions. Eur J Org Chem 2008;35:5946-51.
26. Joshi Krishna C. Preparation of new fluorine containing 2-phenylindole derivatives as antifertility agents. J Indian Chem Soc 1985;62:388-90.
27. Dalton L, Humhrey GL, Cooper MM, Joule JA. Indole (beta)nucleophilic substittution Part-7:(beta)Halogenation of indles attempted intermolecular (beta)nucleophilic substittution of (alpha)arylindoles. J Chem Soc Perkin Trans 1 1983;10:2417-22.