Porous CuO catalysed green synthesis of some novel 3-alkylated indoles as potent antitubercular agents

Gulzar A. Khan\textsuperscript{a}, Javeed A. War\textsuperscript{b}, Gowhar A. Naikoo\textsuperscript{c}, Umar J. Pandit\textsuperscript{a}, Ratnesh Das\textsuperscript{a,*}

\textsuperscript{a} Heterocyclic Synthesis and Electroanalytical Laboratory, Department of Chemistry, Dr. HariSingh Gour Central University, Sagar, India
\textsuperscript{b} Synthetic Organic Chemistry & Molecular Modelling Laboratory, Department of Chemistry, Dr. HariSingh Gour Central University, Sagar, India
\textsuperscript{c} Department of Mathematics and Sciences, College of Arts and Applied Sciences, Dhofar University, Oman

Received 25 January 2016; revised 15 March 2016; accepted 28 March 2016

KEYWORDS
3-Alkylated indoles; Macroporous copper oxide; Green synthesis; Antitubercular activity; Molecular docking

Abstract A green multicomponent one pot synthesis of novel 2-(1H-indol-3-ylmethyl)-5,5-dimethyl-cyclohexane-1,3-diones (4a–l) in excellent yields was conveniently carried out in aqueous medium at room temperature over mpCuO as heterogeneous catalyst. The synthesised 3-alkylated indoles were characterised by FTIR, \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and HRLCMS. The nanocatalyst was facially synthesised via a green sol–gel route and characterised by SEM, TEM, EDX, PXRD, BET and FTIR. The porous nanocatalyst can be recycled five times without significant drop in product yield. Docking studies against enoyl acyl carrier protein reductase predicts that the compounds bind at the active site with high binding affinity values. The compound 4k (MIC, 15 \textmu g/mL) shows comparable activity in reference to Isoniazid at the same concentrations against MT H37 Rv.

1. Introduction

Natural products are the main source of motivation to design and synthesise new molecules for drug development. Most of these natural products consist of a heterocyclic core. Amongst nitrogen containing heterocycles, indole is a ubiquitous structural unit of a number of natural products like rutaecarpine, horsfiline, spirotryprostatin B, cryptosanguinolentine etc. Indole moiety has been employed in the designing of new heterocyclic compounds with diverse biological and pharmacological properties like antimicrobial, antitubercular, antimalarial,
antitubulin, sodium-glucose cotransporter-2 inhibitors, antioxidant and fluorescent metal probes to sense molecular recognitions [1–7]. However, indole derivatives bearing ferrocene moiety and carboxylate chains have been found to exhibit potent anticancer, cytotoxic and antiviral [1,8–11] properties.

Multi-component reactions (MCRs) are of immense importance in the field of medicinal chemistry. MCRs favour molecular diversity to be generated by mixing simple precursors by facile creation of several new bonds in a single-step transformation with no need of intermediate isolation and their purification quite ultimately affords a desirable complex product [9,11–13]. High atom-economy, mild conditions, structural complexity and environmentally benign synthesis of some valuable heterocyclic scaffolds are one of the most advantageous features encountered in MCRs [14].

Heterogeneous catalysis in water as a solvent is regarded as green and sustainable approach. As a consequence, synthetic chemists prefer to design MCRs in water over potentially toxic non-aqueous organic solvents to reduce chemical impurity, to allow easy work-up as a consequence of its ideal behaviour. Water is known to enhance the rate and selectivity of organic transformations due to the interactions like hydrogen bonding, hydrophobic effect and trans-phase interactions [15,16]. Water also tends to have many practical and economic advantages as a reaction solvent, including low cost, inflammable and high specific heat capacity which renders it one of the safest mediums specifically for exothermic transformations [17].

The fusion of a benign aqueous medium and nano-catalyst seems to be a fascinating way to explore the next generation of green and efficient protocols. Because of its high surface area and maximum active sites, nanomaterials exhibit excellent catalytic activities compared with the corresponding bulk materials [18]. In this regard, the searching for a method to incorporate moieties like indole and dimedone to construct a valuable heterocyclic scaffold by nanocatalytic one pot reaction in water should be strongly desired.

The synthesis of 3-substituted indoles via Knoevenagel/ Michael reaction is found to be atom efficient and thus are inherently green transformations [19]. The reaction has been traditionally accomplished with various basic catalysts/reagents, like NaOH [20], and Mg–Al–O–t–Bu hydrotalcite [21]. Under these strong basic conditions side reactions, such as aldol addition, polymerizations, and rearrangements, were frequently observed [22]. Recently, various Lewis and Bronsted acid catalysts such as InBr₃ [23], [Al(DS)₃]₃H₂O [24], FeCl₃ [25], CeCl₃·7H₂O–NaI [26], SmI₃ [27], and K₁₀–FeO [28], and metal salts have been used [29]. However, some of the reported protocols with homogeneous catalysts suffer from severe disadvantages such as strong acidic conditions, complicated work-up procedures, expensive reagents, inadequate yields, and long reaction time.

MCRs assisted by recyclable nanocatalysts under green reaction conditions present an efficient tool for the sustainable synthesis of heterocycles [30]. Nanocrystalline metal oxides of Zn and Mg have been used to catalyse green organic transformations in water [13,31]. However, it is interesting to note that the addition of low levels of Cu as a promoter to ZnS nanoparticles (ZnS NPs) has improved the catalytic activity sevenfold higher than ZnS NPs alone [17]. Doping with copper introduced measurable changes in the surface and catalytic system of a ZnS NPs. Still, there is a demand of developing truly recyclable heterogeneous catalyst under benign reaction conditions.

With this background, and as a part of our on-going research towards green chemistry and nanocatalysis, we herein report a facile one-pot synthesis of a novel series of 2-(1H-indol-3-ylmethyl)-5,5-dimethyl-cyclohexane-1,3-diones 4a–l (Scheme 1) using mpCuO as a reusable heterogeneous catalyst in water. The antitubercular nature of synthesised compounds was determined by means of disc diffusion assay. Amongst the various targets for anti TB drugs, enoyl-acetyl carrier protein (ACP) reductase being a well established target [32,33] was selected as the target for docking simulations. ACP reductase is a key enzyme for the synthesis of the type II fatty acids.

2. Experimental

2.1. Chemicals and apparatus

Sigma–Aldrich, CDH and Merck purchased chemicals were used without purification to carry out this work. Melting points were determined using open capillary tube melting point apparatus and are presented without any correction. The infrared (IR) spectra were recorded on a FTFIR Shimadzu-8400S spectrometer using KBr pellets. The ¹H NMR and ¹³C NMR spectra were recorded on Bruker Avance 400 spectrometer using tetramethylsilane (TMS) as the internal standard and CDCl₃ as solvent. HRLCMS were determined on Bruker Microtuff-QII 10330 mass spectrometer. The purity of the compounds was checked by TLC using Merck pre-coated silica gel GF aluminium plates.

2.2. General one pot synthesis of 2-(1H-indol-3-ylmethyl)-5,5-dimethyl-cyclohexane-1,3-diones (4a–l)

A mixture of indole (1 mmol), dimedone (1 mmol) and substituted aldehyde (1 mmol) in 10 ml water in the presence of 0.04 mmol mpCuO (Supplementary S1–S6) as a heterogeneous catalyst was stirred at room temperature till the completion of reaction (checked by TLC). The resulting solid compound was filtered off and then treated with DMF. The mixture was centrifuged at 2500 rpm for some time to recover mpCuO particles. The organic solution was then poured into water, filtered and recrystallized from ethanol followed by drying under vacuum to afford the pure product (Scheme 1).

2.2.1. 2-{(4-Chloro-phenyl)-(1H-indol-3-ylmethyl)-5,5-dimethyl-cyclohexane-1,3-dione (4a)

FTIR (KBr) (Vₚmax cm⁻¹): 3722 (N–H), 3045 (CH), 1717 (C=O), 1547 (C=C), 1320 (C–N), 822 (C–Cl). ¹H NMR (400 MHZ, CDCl₃, δ ppm): 1.2 (s, 6H, CH₃), 2.4 (s, 4H, CH₂), 3.7 (s, 1H, CH), 4.6 (s, 1H, CH), 6.3 (s, 1H, H$_{Ar}$), 6.9 (d, $J = 8$ Hz, 2H, H$_{Ar}$), 7.0 (dd, $J_1 = 8$ Hz & $J_2 = 7.2$ Hz, 1H, H$_{Ar}$), 7.2 (dd, $J_1 = 7.2$ Hz & $J_2 = 6.8$ Hz, 1H, H$_{Ar}$), 7.5 (d, $J = 3.6$ Hz, 2H, H$_{Ar}$), 7.6 (d, $J = 6.4$ Hz, 1H, H$_{Ar}$), 7.8 (d, $J = 10.4$ Hz, 1H, H$_{Ar}$), 9.4 (s, 1H, NH); ¹³C NMR (125 MHZ, CDCl₃, δ ppm) δₐ: 17.9, 23.9, 36.9, 58.8, 68.7, 109.3, 111.3, 115.3, 116.6, 116.8, 117.84, 118.8,
2.2.2. 2-[(1H-indol-3-yl)-phenyl-methyl]-5,5-dimethyl-cyclohexane-1,3-dione (4b)

**FTIR** (KBr) ($V_{\text{max}}$ cm$^{-1}$): 3722 (N–H), 3073 (CH), 1707 (C=O), 1537 (C=C), 1320 (C–N). $^1$H NMR (400 MHz, CDCl$_3$, TMS, $\delta$ ppm): 1.2 (s, 6H, CH$_3$), 2.4 (s, 4H, CH$_2$), 3.7 (s, 1H, CH), 4.6 (s, 1H, CH), 6.3 (s, 1H, H$_{Ar}$), 6.8 (m, 5H, H$_{Ar}$), 7.0 (dd, $J_1 = 8$ Hz & $J_2 = 7.2$ Hz, 1H, H$_{Ar}$), 7.2 (dd, $J_1 = 7.2$ Hz & $J_2 = 6.8$ Hz, 1H, H$_{Ar}$), 7.6 (d, $J = 6.4$ Hz, 1H, H$_{Ar}$), 7.8 (d, $J = 10.4$ Hz, 1H, H$_{Ar}$), 9.4 (s, 1H, NH); $^{13}$C NMR (125 MHz, CDCl$_3$, $\delta$ ppm) $\delta_{c}$: 17.9, 23.9, 36.9, 58.8, 68.7, 109.3, 111.3, 115.3, 116.6, 116.8, 117.84, 118.8, 121.1, 122.2, 124.4, 128.3, 131.1, 145, 169.7; HRLCMS (ESI): $m/z$ [M + H]$^+$: 346.1812.

2.2.3. 2-[(3-Nitro-phenyl)-(1H-indol-3-yl)-methyl]-5,5-dimethyl-cyclohexane-1,3-dione (4c)

**FTIR** (KBr) ($V_{\text{max}}$ cm$^{-1}$): 3722 (N–H), 3070 (CH), 1707 (C=O), 1546 (C=C), 1371 (N=O), 1320 (C–N). $^1$H NMR (400 MHz, CDCl$_3$, TMS, $\delta$ ppm): 1.2 (s, 6H, CH$_3$), 2.4 (s, 4H, CH$_2$), 3.7 (s, 1H, CH), 4.6 (s, 1H, CH), 6.3 (s, 1H, H$_{Ar}$), 7.0 (dd, $J_1 = 8$ Hz & $J_2 = 7.2$ Hz, 1H, H$_{Ar}$), 7.2 (dd, $J_1 = 7.2$ Hz & $J_2 = 6.8$ Hz, 1H, H$_{Ar}$), 7.4 (dd, $J = 4.6$ Hz, 1H, H$_{Ar}$), 7.5 (d, $J = 7.6$ Hz, 1H, H$_{Ar}$), 7.6 (d, $J = 6.4$ Hz, 1H, H$_{Ar}$), 7.8 (d, $J = 10.4$ Hz, 1H, H$_{Ar}$), 9.4 (s, 1H, NH); $^{13}$C NMR (125 MHz, CDCl$_3$, $\delta$ ppm) $\delta_{c}$: 17.9, 23.9, 36.9, 58.8, 68.7, 109.3, 111.3, 115.3, 115.7, 116.6, 116.8, 117, 117.84, 118.8, 128.3, 130, 131.1, 143.4, 143.5, 145.7, 147, 169.7; HRLCMS (ESI): $m/z$ [M + H]$^+$: 328.1712.

2.2.4. 2-[(4-Nitro-phenyl)-(1H-indol-3-yl)-methyl]-5,5-dimethyl-cyclohexane-1,3-dione (4d)

**FTIR** (KBr) ($V_{\text{max}}$ cm$^{-1}$): 3722 (N–H), 3078 (CH), 1707 (C=O), 1558 (C=C), 1371 (N=O), 1320 (C–N). $^1$H NMR (400 MHz, CDCl$_3$, TMS, $\delta$ ppm): 1.2 (s, 6H, CH$_3$), 2.4 (s, 4H, CH$_2$), 3.7 (s, 1H, CH), 4.6 (s, 1H, CH), 6.3 (s, 1H, H$_{Ar}$), 7.0 (dd, $J_1 = 8$ Hz & $J_2 = 7.2$ Hz, 1H, H$_{Ar}$), 7.2 (dd, $J_1 = 7.2$ Hz & $J_2 = 6.8$ Hz, 1H, H$_{Ar}$), 7.4 (d, $J = 6.8$ Hz, 2H, H$_{Ar}$), 7.6 (d, $J = 6.4$ Hz, 1H, H$_{Ar}$), 7.8 (d, $J = 10.4$ Hz, 1H, H$_{Ar}$), 8.1 (d, $J = 4.4$ Hz, 2H, H$_{Ar}$), 9.4 (s, 1H, NH); $^{13}$C NMR (125 MHz, CDCl$_3$, $\delta$ ppm) $\delta_{c}$: 17.9, 23.9, 36.9, 58.8, 68.7, 109.3, 111.3, 115.3, 116.5, 116.8, 117.84, 118.8, 124.7, 126.7, 128.3, 131.1, 147, 169.7; HRLCMS (ESI): $m/z$ [M + H]$^+$: 391.1712.
6.8 (m, 5H, H Ar), 7.3 (d, J = 3.2 Hz, 1H, H Ar), 7.4 (d, J = 6.8 Hz, 1H, H Ar), 7.7 (s, 1H, H Ar), 9.4 (s, 1H, NH); 13C NMR (125 MHz, CDCl 3, δ ppm): 7.6 (m, 5H, H Ar), 7.7 (s, 1H, H Ar), 9.4 (s, 1H, NH); 13C NMR (125 MHz, CDCl 3, δ ppm): 17.9, 23.9, 36.9, 58.8, 68.7, 111.3, 116, 116.3, 120, 121, 122.3, 123.4, 124, 124.4, 128, 131.8, 145, 169.7; HRLCMS (ESI): m/z [M + H]+: 424.0912.

2.2.9. 2-{[5-Bromo-1H-indol-3-yl]-[3-Nitro-phenyl]-methyl]-5,5-dimethyl-cyclohexane-1,3-dione (4j)

FTIR (KBr) (V max cm⁻¹): 3722 (N–H), 3081 (CH), 1707 (C=O), 1546 (C=C), 1371 (N–O), 1320 (C–N), 658 (C–Br). 1H NMR (400 MHz, CDCl 3, TMS, δ ppm): 1.2 (s, 6H, CH 3), 2.4 (s, 4H, CH 2), 3.7 (s, 1H, CH), 4.6 (s, 1H, CH), 6.3 (s, 1H, H Ar), 7.3 (d, J = 3.2 Hz, 1H, H Ar), 7.4 (d, J = 6.4 Hz, 2H, H Ar), 7.4 (d, J = 6.8 Hz, 1H, H Ar), 7.7 (s, 1H, H Ar), 7.9 (d, J = 3.2 Hz, 1H, H Ar), 8.0 (d, J = 7.6 Hz, 2H, H Ar), 9.4 (s, 1H, NH); 13C NMR (125 MHz, CDCl 3, δ ppm): 17.9, 23.9, 36.9, 58.8, 68.7, 111.3, 115.7, 116, 116.3, 117, 120, 123.4, 124, 128, 130, 131.8, 143.4, 143.5, 145.7, 147, 169.7; HRLCMS (ESI): m/z [M + H]+: 470.0812.

2.2.10. 2-{[5-Bromo-1H-indol-3-yl]-[4-Nitro-phenyl]-methyl]-5,5-dimethyl-cyclohexane-1,3-dione (4j)

FTIR (KBr) (V max cm⁻¹): 3722 (N–H), 3075 (CH), 1707 (C=O), 1546 (C=C), 1371 (N–O), 1320 (C–N), 658 (C–Br). 1H NMR (400 MHz, CDCl 3, TMS, δ ppm): 1.2 (s, 6H, CH 3), 2.4 (s, 4H, CH 2), 3.7 (s, 1H, CH), 4.6 (s, 1H, CH), 6.3 (s, 1H, H Ar), 7.3 (d, J = 3.2 Hz, 1H, H Ar), 7.4 (d, J = 6.4 Hz, 2H, H Ar), 7.4 (d, J = 6.8 Hz, 1H, H Ar), 7.7 (s, 1H, H Ar), 8.1 (d, J = 4.4 Hz, 2H, H Ar), 9.4 (s, 1H, NH); 13C NMR (125 MHz, CDCl 3, δ ppm): 17.9, 23.9, 36.9, 45.5, 58.8, 68.7, 111.3, 116, 116.3, 120, 123.4, 124, 124.7, 126.7, 128, 131.8, 147, 169.7; HRLCMS (ESI): m/z [M + H]+: 470.0812.

2.2.11. 2-{[5-Bromo-1H-indol-3-yl]-[4-Methoxy-phenyl]-methyl]-5,5-dimethyl-cyclohexane-1,3-dione (4k)

FTIR (KBr) (V max cm⁻¹): 3725 (N–H), 3120 (CH), 1715 (C=O), 1480 (C=C), 822 (C=C), 717 (C–Br). 1H NMR (400 MHz, CDCl 3, TMS, δ ppm): 1.2 (s, 6H, CH 3), 2.4 (s, 4H, CH 2), 3.6 (s, 3H, CH 3), 3.7 (s, 1H, CH), 4.6 (s, 1H, CH), 6.1 (d, J = 4.4 Hz, 2H, H Ar), 6.3 (s, 1H, H Ar), 7.01 (d, J = 7.6 Hz, 2H, H Ar), 7.3 (d, J = 3.2 Hz, 1H, H Ar), 7.4 (d, J = 6.8 Hz, 1H, H Ar), 7.7 (s, 1H, H Ar), 8.1 (d, J = 4.4 Hz, 2H, H Ar), 9.4 (s, 1H, NH); 13C NMR (125 MHz, CDCl 3, δ ppm): 17.9, 23.9, 36.9, 45.5, 58.8, 68.7, 111.3, 114, 116.3, 120, 123.4, 124, 124.7, 126.7, 128, 131.8, 159.2 169.7; HRLCMS (ESI): m/z [M + H]+: 454.1012.

2.2.12. 2-{[5-Bromo-1H-indol-3-yl]-ethyl}-5,5-dimethyl-cyclohexane-1,3-dione (4l)

FTIR (KBr) (V max cm⁻¹): 3513 (OH), 3151 (N–O), 3026 (CH), 1700 (C=O), 1645 (C=C), 1348 (C–O), 1238 (C–N). 1H NMR (400 MHz, CDCl 3, TMS, δ ppm): 1.2 (s, 6H, CH 3), 1.4 (s, 3H, CH 3), 2.4 (s, 4H, CH 2), 3.7 (s, 1H, CH), 4.6 (s, 1H, CH), 6.3 (s, 1H, H Ar), 7.3 (d, J = 3.2 Hz, 1H, H Ar), 7.4 (d, J = 6.8 Hz, 1H, H Ar), 7.7 (s, 1H, H Ar), 9.4 (s, 1H, NH); 13C NMR (125 MHz, CDCl 3, δ ppm): 15.3, 17.9, 21, 23.9, 58.8, 68.7, 109, 111, 116, 116.3, 120, 123.4, 124, 128, 131.8; HRLCMS (ESI): m/z [M + H]+: 362.0812.

2.3. In vitro biological evaluation assay

2.3.1. Anti tubercular activity

Mycobacterium tuberculosis bacterial strain (MTCC CODE 300) purchased from the Institute of Microbial Technology (MTCC) Chandigarh (India), was cultured in blood nutrient agar medium in late logarithmic (A 600 nm = 1) fashion. Bacterial strains possessing a plasmid adhering Isoniazid resistance marker were cultured in the same medium containing 100 μg/mL Isoniazid. It was shortly followed by streaking of 0.5 μL bacterial spread on LB agar plates (25 mL agar medium ± 90 μg/mL Isoniazid discs over 9 cm Petri plates) as control. Filter discs (5 mm diameter) of Whatman range were treated with 5 μL of compound solutions including reference (Isoniazid). After this, discs were air-dried for 7–10 min and kept over the plates. These plates were incubated at 37 °C for about 48 h in a humid chamber [34]. Following this, zone inhibition diameters were observed and measured carefully.

2.4. Docking study

The high resolution crystal structure of Enoyl-ACP reductase was downloaded from the RCSB PDB website (PDB ID: 1QG6) [35]. All molecular docking calculations were performed on Auto Dock-Vina software [36]. The protein was prepared for docking by removing the co-crystallized ligands, waters and co-factors. The AutoDockTools (ADT) graphical user interface was used to calculate Kollman charges and polar hydrogens. The ligand was prepared for docking by minimizing its energy with MMFF94 s force field. Partial charges were calculated by Geistenger method. The active site of the enzyme was defined to include residues of the active site within the grid size of 40 Å × 40 Å × 40 Å. The most popular algorithm, Lamarckian Genetic Algorithm (LGA) available in Autodock was employed for docking. The docking protocol was tested by extracting co-crystallized an inhibitor from the protein and then docking the same. The docking protocol predicted the same conformation as was present in the crystal structure with RMSD value well within the reliable range of <1 Å (Fig. 1). Amongst the docked conformations, one which

Figure 1 Superimposition of docked conformation (pink) over the co-crystallized conformation (yellow) of triclosan shows RMSD value close to zero, confirming the reliability of docking protocol.
bonds well as the active site was analysed for detailed interactions in Discover Studio Visualizer 4.

3. Results and discussion

3.1. Synthesis

To the best of our knowledge, this new synthetic strategy provides the first example of a facile three component protocol for the preparation of novel 2-(1H-indol-3-ylmethyl)-5,5-dimethyl-cyclohexane-1,3-diones (4a–l). To get the best experimental conditions, the reaction conditions were optimised for the preparation of 4a chosen as model reaction for both at room temperature and at reflux conditions. As depicted in Scheme 1 indole and dimedone as reacting materials were treated with different aldehydes. A number of solvents in the presence of a variety of catalysts (with/without suitable additive) were employed to design a desirable reaction medium. Initially dichloroethane (DCE), a nonpolar solvent in the presence of anh.FeCl₃ furnished 4a with poor yield including prolonged reaction time (Table 1; entry 1, 23%) [25]. Afterwards, reaction was performed in solvents like dimethyl sulphoxide (DMSO), acetonitrile (ACN), tetrahydrofuran (THF/HOH) and dimethyl formamide (DMF) using different metal catalysts like CuI/K₂CO₃, KOtBu, Li(OH)₂, and Cs₂CO₃. It was observed that the product yield was obtained in 9%, 18%, 11%, 14%, and 23% (Table 1; entry 2–6) but prolonged reaction time was still a challenge [9,37,38,19,39]. Inspired by these results the reaction was then carried in more polar solvents like methanol (MeOH), ethanol (EtOH/HOH), acetic acid (AcOH) and water using the catalysts anh.ZnCl₂, AcOH, 4-dimethylaminopyridine (DMAP), dibutylamine and PEG/OSO₃H. In this context, the reaction occurred smoothly and improved product yield as well as reaction time. As a result we were able to isolate the product yield at 27%, 33%, 43%, 57%, 43% and 55% (Table 1; entry 7–12) [40–45]. Finally, water was taken as a best medium choice which in the presence of a suitable catalyst mpCuO (Table S1, 4 mol %) afforded 4a (Table 1; entry 13, 93%) in highest yield than all other solvents. It is evident that amongst these catalysts, mpCuO give the better results. Besides interesting features regarding high surface area, nice porosity, moisture stability, the better catalytic activity of mpCuO may also be attributed to high proportion of structural defects and minimal coordinated sites on its surface [46,47].

The plausible mechanism (Scheme 2) explaining the aforementioned results assumes that mpCuO coordinates to the carbonyl oxygen of aldehyde and facilitates its reaction with dimedone via Knoevenagel type coupling results in the formation of \( \alpha, \beta \)-unsaturated ketone 6. Coordination of mpCuO to first formed Knoevenagel product 6 activates it towards nucleophilic addition of indole at its C(3) position to afford the intermediate 7. Finally, there occurs electron reorganization in 7 accompanied with H-transfer to afford desirable compound 4, acquitting the catalyst for the next cycle (see Table 2).

The reusability of the catalysts broadens their area of potential applications. It is pertinent to mention that the mpCuO was found to exhibit almost same catalytic activity only showing a slight variation in reaction rate and % yield of desired product. It may be due to coagulation of the nano

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Additive</th>
<th>Catalyst</th>
<th>Time (h)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCE</td>
<td>–</td>
<td>Anh.FeCl₃</td>
<td>P 11</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>DMSO</td>
<td>K₂CO₃</td>
<td>CuI</td>
<td>P 10</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>DMSO</td>
<td>KOtBu</td>
<td>P 15</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ACN</td>
<td>–</td>
<td>Q 19</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>THF/HOH</td>
<td>–</td>
<td>Li(OH)₂</td>
<td>Q 19</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>DMF</td>
<td>–</td>
<td>Cs₂CO₃</td>
<td>Q 9</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>MeOH</td>
<td>–</td>
<td>Q 7</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>MeOH</td>
<td>–</td>
<td>Anh.ZnCl₂</td>
<td>Q 12</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>EtOH/HOH</td>
<td>–</td>
<td>DMAP</td>
<td>Q 10</td>
<td>31</td>
</tr>
<tr>
<td>10</td>
<td>EtOH</td>
<td>–</td>
<td>P 5</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>AcOH</td>
<td>–</td>
<td>Q 11</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>HOH</td>
<td>–</td>
<td>P 8</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>HOH</td>
<td>–</td>
<td>P 0.33</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

PReflux condition, Qroom temperature.

Please cite this article in press as: G.A. Khan et al., Porous CuO catalysed green synthesis of some novel 3-alkylated indoles as potent antitubercular agents, Journal of Saudi Chemical Society (2016), http://dx.doi.org/10.1016/j.jscs.2016.03.009
particles resulting in the reduction of surface area of nanocatalyst used (Fig. 2). The catalyst recovered above was added to the aqueous phase containing the same reactants. The experiments were repeated for four times and the products were identified by their melting points and IR spectra. As indicated in the Fig. 2, the catalyst can be used more than five times without a significant drop in product yield.

The synthesised compounds 4a–l were characterised by different spectral techniques like IR, NMR and HRLCMS. IR spectrum of compound 4a exhibited sharp bands at 822 cm\(^{-1}\) and 1320 cm\(^{-1}\) assigned to C–Cl and C–N stretching frequencies respectively. The band at 1717 cm\(^{-1}\) indicated the presence of dimedone carbonyl whereas aromatic C–H stretching was observed at 3045 cm\(^{-1}\). In \(^1\)H NMR spectrum of 4a four singlets due to protons of C15/16-CH\(_3\), C12/14-CH\(_2\), C13-CH\(_3\), and C14-CH\(_3\) were observed.

### Table 2 Water mediated one pot synthesis of 2-(1H-indol-3-ylmethyl)-5,5-dimethyl-cyclohexane-1,3-diones (4a–l).

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>R’</th>
<th>Protocol</th>
<th>Time (min)</th>
<th>Product</th>
<th>(% Yield isolated)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p-ClC(_6)H(_5)</td>
<td>H</td>
<td>P</td>
<td>20</td>
<td>4a</td>
<td>93</td>
<td>133–136</td>
</tr>
<tr>
<td>2</td>
<td>C(_6)H(_5)</td>
<td>H</td>
<td>P</td>
<td>15</td>
<td>4b</td>
<td>91</td>
<td>141–144</td>
</tr>
<tr>
<td>3</td>
<td>m-NO(_2)C(_6)H(_5)</td>
<td>H</td>
<td>P</td>
<td>23</td>
<td>4c</td>
<td>85</td>
<td>139–142</td>
</tr>
<tr>
<td>4</td>
<td>p-NO(_2)C(_6)H(_5)</td>
<td>H</td>
<td>P</td>
<td>20</td>
<td>4d</td>
<td>89</td>
<td>127–130</td>
</tr>
<tr>
<td>5</td>
<td>p-OMeC(_6)H(_5)</td>
<td>H</td>
<td>P</td>
<td>22</td>
<td>4e</td>
<td>95</td>
<td>152–155</td>
</tr>
<tr>
<td>6</td>
<td>CH(_3)</td>
<td>H</td>
<td>P</td>
<td>17</td>
<td>4f</td>
<td>82</td>
<td>135–138</td>
</tr>
<tr>
<td>7</td>
<td>p-ClC(_6)H(_5)</td>
<td>Br</td>
<td>P</td>
<td>23</td>
<td>4g</td>
<td>90</td>
<td>146–149</td>
</tr>
<tr>
<td>8</td>
<td>C(_6)H(_5)</td>
<td>Br</td>
<td>P</td>
<td>19</td>
<td>4h</td>
<td>83</td>
<td>139–142</td>
</tr>
<tr>
<td>9</td>
<td>m-NO(_2)C(_6)H(_5)</td>
<td>Br</td>
<td>P</td>
<td>27</td>
<td>4i</td>
<td>85</td>
<td>126–129</td>
</tr>
<tr>
<td>10</td>
<td>p-NO(_2)C(_6)H(_5)</td>
<td>Br</td>
<td>P</td>
<td>23</td>
<td>4j</td>
<td>88</td>
<td>162–165</td>
</tr>
<tr>
<td>11</td>
<td>p-OMeC(_6)H(_5)</td>
<td>Br</td>
<td>P</td>
<td>31</td>
<td>4k</td>
<td>91</td>
<td>169–172</td>
</tr>
<tr>
<td>12</td>
<td>CH(_3)</td>
<td>Br</td>
<td>P</td>
<td>20</td>
<td>4l</td>
<td>78</td>
<td>144–147</td>
</tr>
</tbody>
</table>

*Room temperature.*

Figure 2: Reusability of the catalyst mpCuO.

Please cite this article in press as: G.A. Khan et al., Porous CuO catalysed green synthesis of some novel 3-alkylated indoles as potent antitubercular agents, Journal of Saudi Chemical Society (2016), http://dx.doi.org/10.1016/j.jscs.2016.03.009
C10-CH and C9-CH were recorded at 1.2, 2.4, 3.7 and 4.6 δ ppm. The proton at C7-position shows a singlet at 6.3 δ ppm whereas resonance emission of protons at C10 and C20 positions appeared as two double doublets at 7.0 and 7.2 δ ppm. The rest of the proton signals were recorded in the expected regions. Also 13C NMR of compound 4a agrees with the number of carbons. It exhibited signals around 17–81 δ ppm attributed to methyl, methylene and methine carbon atoms. Peaks between 109–143 δ ppm were assigned to aromatic carbon atoms and a peak at 169 δ ppm might be due to dimedone carbonyl atom. The unambiguous confirmation of 4a was done from its mass spectra exhibited the molecular ion peak at m/z 380.1412 [M + 1]+, in agreement with the molecular weight of the compound (Supplementary S7–S9).

3.2. Docking study

Docking study reveals that the synthesised molecules bind at the same site where the co-crystallized drug Triclosan is attached. (Fig. 3) Ser91 and Thr194 form H-bonds of length 2.84 and 2.78 Å respectively with 4k, the highest scoring molecule (Fig. 4). These interactions along with other prominent interactions are described in (Fig. 5). The interactions predicted by docking study suggest a strong ligand-macromolecule complex. These findings reveal that the synthesised molecules possess high affinity towards bacterial Enoyl-ACP reductase. Binding affinity values of greater than −9 kcal/mol for all the ligand-ACP-reductase complexes corroborates the above conclusion (Table S2). Compound 4e, 4f and 4a follow compound 4k in docking score. The computational predictions were complemented by the in vitro antitubercular activity values.

As is evident from Table 3, compound 4d, 4e, 4j and 4k are able to inhibit the bacterial strain to appreciable levels with respective MIC values of 60, 15, 15 and 60 μg/mL in comparison to Isoniazid taken as standard against Mycobacterium tuberculosis. However the rest of the tested compound showed MIC values ranging from 90 to > 180 μg/mL (Table 3). In general the methoxy and nitro derivatives show better activity as compared to chloro, methyl and benzyl derivatives. The reason behind the observed activity trend seems to be the way in which the molecules bind at the target active site as is predicted by docking (Figs. 1 and 3). The compound 4k shows better inhibition than all other compounds at the same concentrations.
4. Conclusion

Here we report the fusion of benign aqueous medium and nano-catalyst to develop a mild and green approach with higher environmental sustainability for the synthesis of novel 2-(1H-indol-3-ylmethyl)-5,5-dimethyl-cyclohexane-1,3-diones employing a MCR protocol. Operational simplicity, reduced reaction times, better functional group tolerance, excellent yields and ease of product isolation are the advantageous features encountered which makes the present methodology as desirable. Attractive features of mpCuO are high catalytic efficiency, maximum surface area and recyclability. In vitro antitubercular evaluation has verified that compounds (4a-l) can be used to generate a library of antitubercular analogues which can exhibit better selectivity. Indeed, docking studies corroborate to the in vitro results as well.

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Binding affinity</th>
<th>logP</th>
<th>H-bond donors</th>
<th>H-bond acceptors</th>
<th>Molecular weight</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>−9.5</td>
<td>5.4453</td>
<td>1</td>
<td>2</td>
<td>380.13</td>
<td>17.31 ± 0.17 &gt; 180</td>
</tr>
<tr>
<td>4b</td>
<td>−9.2</td>
<td>5.9443</td>
<td>1</td>
<td>2</td>
<td>346.18</td>
<td>15.84 ± 0.16 &gt; 180</td>
</tr>
<tr>
<td>4c</td>
<td>−9.3</td>
<td>6.4733</td>
<td>1</td>
<td>3</td>
<td>391.17</td>
<td>11.54 ± 0.38 &gt; 180</td>
</tr>
<tr>
<td>4d</td>
<td>−9.0</td>
<td>6.3291</td>
<td>1</td>
<td>3</td>
<td>391.17</td>
<td>22.11 ± 0.03 60</td>
</tr>
<tr>
<td>4e</td>
<td>−9.9</td>
<td>5.5543</td>
<td>1</td>
<td>3</td>
<td>376.19</td>
<td>24.39 ± 0.26 30</td>
</tr>
<tr>
<td>4f</td>
<td>−9.6</td>
<td>5.5543</td>
<td>1</td>
<td>2</td>
<td>284.17</td>
<td>21.38 ± 0.04 90</td>
</tr>
<tr>
<td>4g</td>
<td>−9.6</td>
<td>3.4713</td>
<td>1</td>
<td>2</td>
<td>460.09</td>
<td>18.27 ± 0.06 &gt; 180</td>
</tr>
<tr>
<td>4h</td>
<td>−9.3</td>
<td>3.9704</td>
<td>1</td>
<td>2</td>
<td>424.09</td>
<td>20.75 ± 0.10 180</td>
</tr>
<tr>
<td>4i</td>
<td>−9.2</td>
<td>4.4993</td>
<td>1</td>
<td>3</td>
<td>470.08</td>
<td>14.19 ± 0.01 &gt; 180</td>
</tr>
<tr>
<td>4j</td>
<td>−9.2</td>
<td>4.3551</td>
<td>1</td>
<td>3</td>
<td>470.08</td>
<td>23.08 ± 0.03 60</td>
</tr>
<tr>
<td>4k</td>
<td>−10.1</td>
<td>3.5803</td>
<td>1</td>
<td>3</td>
<td>454.10</td>
<td>25.14 ± 0.06 15</td>
</tr>
<tr>
<td>4l</td>
<td>−9.0</td>
<td>3.5803</td>
<td>1</td>
<td>2</td>
<td>362.08</td>
<td>20.68 ± 0.07 90</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>−7.3</td>
<td>−0.69</td>
<td>2</td>
<td>2</td>
<td>137.14</td>
<td>25.70 ± 0.05 10</td>
</tr>
</tbody>
</table>

* Average of three.
Acknowledgement

The authors are thankful to Mr. A. Kumar Department of Zoology, Dr. Hari Singh Gour Central University, Sagar (M.P) for his help in carrying out antitubercular assay. GAK, and UJP acknowledges UGC, New Delhi for financial support as Central University Fellowship (CUF). JAW acknowledges the DST, New Delhi for INSPIRE fellowship (IF-120399). Authors are thankful to Prof. T.S.S. Rao, Dr. Rampal Pandey, Dr. K.B. Joshi and Dr. K. Das for their time to time nice suggestions regarding the present work. Authors are also thankful to SIL of Dr. Hari Singh Gour Central University, Sagar (M.P) and IISER Bhopal, India for providing instrumental facilities.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jscs.2016.03.009.

References


[18] D.N. Komm, D. Kumar, A.K. Chakraborty, All water chemistry for a concise total synthesis of the novel class antianginal drug (RS), (R), and (S)-ranolazine, Green Chem. 15 (2013) 756.


J. Safari, Z. Zarnegar, M. Heydarian, Practical, ecofriendly, and highly efficient synthesis of 2-amino-4H-chromenes using nanocrystalline MgO as a reusable heterogeneous catalyst in aqueous media.


C. Han, W. Meng, H. Liu, Y. Liu, J. Tao, DMAP-catalyzed four-component one-pot synthesis of highly functionalized spiroxindole-1,4-dihydropyridines derivatives in aqueous ethanol, Tetrahedron 70 (45) (2014) 8678–8674.


