Synthesis, antimicrobial activity, pharmacophore modeling and molecular docking studies of new pyrazole-dimedone hybrid architectures

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Abstract

Background: Design and synthesis of pyrazole-dimedone derivatives were described by one-pot multicomponent reaction as new antimicrobial agents. These new molecular framework were synthesized in high yields with a broad substrate scope under benign conditions mediated by diethylamine (NHEt₂). The molecular structures of the synthesized compounds were assigned based on different spectroscopic techniques (¹H-NMR, ¹³C-NMR, IR, MS, and CHN).

Results: The synthesized compounds were evaluated for their antibacterial and antifungal activities against S. aureus ATCC 29213, E. faecalis ATCC29212, B. subtilis ATCC 10400, and C. albicans ATCC 2091 using agar Cup plate method. Compound 4b exhibited the best activity against B. subtilis and E. faecalis with MIC = 16 µg/L. Compounds 4e and 4l exhibited the best activity against S. aureus with MIC = 16 µg/L. Compound 4k exhibited the best activity against B. subtilis with MIC = 8 µg/L. Compounds 4o was the most active compounds against C. albicans with MIC = 4 µg/L.

Conclusion: In-silico predictions were utilized to investigate the structure activity relationship of all the newly synthesized antimicrobial compounds. In this regard, a ligand-based pharmacophore model was developed highlighting the key features required for general antimicrobial activity. While the molecular docking was carried out to predict the most probable inhibition and binding mechanisms of these antibacterial and antifungal agents using the MOE docking suite against few reported target proteins.

Keywords: Pyrazole, Dimedone, Antifungal activity, Antimicrobial activity, Structure activity relationship, Inhibition mechanism prediction

Background

Nosocomial infections caused by antibiotic-resistant gram-positive bacteria have become a serious medical problem with an alarming increasing rate worldwide. Methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE) and penicillin-resistant Streptococcus pneumoniae (PRSP) are of particular concern among various hospital-acquired infections [1]. Accordingly, emerging investigations have provided new insights into developing novel, safe and effective antibacterial agents. Within this scope, pyrazole based antibacterial agents attracted great interest [2]. Generally, pyrazoles display innumerable pharmacological activities ranging from analgesic, antipyretic, antimicrobial, anti-inflammatory, anticonvulsant, and selective enzyme inhibitory activities [2–11]. Recently, Barakat et al, have been reported novel pyrazole hybrid architectures as efficient antibacterial agents. Various pharmacophores were linked to the pyrazole core to build bioactive scaffolds [12, 13]. Within this approach, cyclic dicarbonyl...
compounds of the type dimedone have attracted our interest. Dimedone has been utilized successfully as pharmacophoric building block in various antimicrobial agents such as xanthenes [14, 15], substituted chromenes [16], macrocyclic metal complexes [17], quinazoline derivatives [18], tetrahydro quinolone diones [19] and acridine based compounds [20]. Recognizing these facts and in continuation of our previous work [12, 13] new hybrid molecules incorporating pyrazoles and dimedone in a single molecular framework were designed and synthesized. We subjected our target compounds to pharmacophore modeling and molecular docking on different target proteins to explore their mode of action.

Results and discussion

Chemistry

The designed bioactive scaffolds were synthesized utilizing green approach. The pyrazole-dimedone derivatives were prepared as shown in Scheme 1 via one pot Knoevenagel condensation Michael addition of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one, 1,3-dicarbonyl compound (dimedone) and various aldehydes mediated by aqueous NH$_2$Et$_2$. This one pot multicomponent reaction afforded the final targets as hybrid frameworks 4a–o in good yields (40–78%) with substrate tolerance of pyrazole-dimedone derivatives. The chemical structures of all the synthesized compounds were assigned by the aid of physical and spectroscopic methods ($^1$H-NMR, $^{13}$C-NMR, IR, and elemental analyses).

The suggested mechanisms for obtaining the target compounds are shown in Scheme 2. Olefin is formed by Knoevenagel condensation of aryl aldehyde 1 and 1,3-diketone 2 to give benzylidenecyclohexandione intermediate which acts as a Michael acceptor. This Michael acceptor is attached by 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one 3 (Michael donor) to give the requisite final targets 4a (Path A). Another bath way is Knoevenagel condensation between aryl aldehyde 1 and 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one 3 to generate benzylidene pyrazolone intermediate which acts as a Michael acceptor. This Michael acceptor is attacked by 1,3-diketone 2 (Michael donor) to afford the final product 4a (Path B).

Antimicrobial activity

The synthesized pyrazole-dimedone derivatives showed various antibacterial activities. Results of the bactericidal activity are shown in Table 1; the minimum inhibitory concentration (MIC) results are expressed as µg/L inhibition.

Antibacterial activity against gram positive bacteria

The antibacterial activity of the novel pyrazole-dimedone compounds were evaluated against gram positive bacteria including E. faecalis ATCC29212, S. aureus ATCC 29213, and B. subtilis ATCC 10400. Ciprofloxacin was used as standard drug.

The results listed in Table 1 revealed that all pyrazole-dimedone compounds were active against the tested-strains including S. aureus, E. faecalis, and B. subtilis. Pyrazole-dimedone 4k was the most active compound against B. subtilis with MIC value of 8 µg/L. Compounds 4e and 4l having 3-methyl and 4-trifluromethyl substituents on the phenyl ring respectively exhibited good activity against S. aureus with MIC value of 16 µg/L. Compounds 4a-d, 4f,g,i,k and 4m-o showed relatively lower activity against S. aureus with MIC value of 32 µg/L. Compounds 4h and 4j having 4-nitro and 4-methoxy substituents on the phenyl ring were the least active derivatives against S. aureus with MIC values of 64 µg/L. Compound 4b bearing unsubstituted phenyl ring exhibited good activity against E. faecalis with MIC values of 16 µg/L. Compounds 4a, c-e, 4g, h and 4j–o showed lower activity against E. faecalis with MIC value of 32 µg/L. Compounds 4f and 4i having 4-bromo and 3-nitro substituents on the phenyl ring respectively were shown as the least active derivatives against E. faecalis with MIC value of 64 µg/L.

Substituted pyrazole-dimedone 4b without substituent on the phenyl ring and 4o having thiophene ring exhibited good activity against B. subtilis with MIC value of 16 µg/L. Compounds 4a, c, d, 4f–j and 4l–o showed lower activity against B. subtilis with MIC value of 32 µg/L. Compound 4e having 3-methyl substituent on the phenyl ring was shown to be the least active against B. subtilis with MIC value of 64 µg/L.

Antifungal activity

The newly synthesized pyrazole-dimedone derivatives were evaluated for their antifungal activity against fungi C. albicans (ATCC 2091) by the diffusion agar and serial dilution method (BSAC, 2015) [23] Fluconazole was used as standard antifungal agent. Results shown in Table 1 revealed that all pyrazole-dimedone compounds 4a-o were active against the tested-strains C. albicans ATCC 2091. Pyrazole-dimedone 4o bearing thiophene was the most active compounds from this series against C. albicans ATCC 2091 with MIC value of 4 µg/L. Compounds 4c, d, h, k, m possessed good activity against C. albicans with MIC values of 16 µg/L. Compounds 4a, b, 4e–g, and 4i, j, g, n were the least active among this series as antifungal agent with MIC values of 32 µg/L.
| #  | 4    | R             | yield (%)<sup>b</sup> |
|----|------|---------------|-----------------------|
| 1  | 4a   | 2,4-Cl<sub>2</sub>Ph | 78                    |
| 2  | 4b   | Ph            | 62                    |
| 3  | 4c   | p-ClPh        | 50                    |
| 4  | 4d   | p-CH<sub>3</sub>Ph | 62                    |
| 5  | 4e   | m-CH<sub>3</sub>Ph | 66                    |
| 6  | 4f   | p-BrPh        | 71                    |
| 7  | 4g   | m-BrPh        | 70                    |
| 8  | 4h   | p-NO<sub>2</sub>Ph | 52                    |
| 9  | 4i   | m-NO<sub>2</sub>Ph | 63                    |
| 10 | 4j   | p-CH<sub>3</sub>OPh | 64                    |
| 11 | 4k   | p-FPh         | 57                    |
| 12 | 4l   | p-CF<sub>3</sub>Ph | 76                    |
| 13 | 4m   | 2,6-Cl<sub>2</sub>Ph | 40                    |
| 14 | 4n   | 2-Naphthaldehyde | 76                    |
| 15 | 4o   | Thiophene     | 75                    |

<sup>a</sup> All reactions were carried out with aldehyde 1 (1.5 mmol), 5,5-dimethylcyclohexane-1,3-dione 2 (1.5 mmol), 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1.5 mmol) and amine (1.5 mmol) in water (1.5 mL) for the specified time. <sup>b</sup> Yield of isolated product.

**Scheme 1** Substrate scope of the cascade reaction: variation of pyrazole-dimedone adducts
Structure activity relationship profiling via pharmacophore modeling
First of all, to predict the structure activity relationship (SAR) of all the newly synthesized antimicrobial compounds, a ligand-based pharmacophore model was developed. This is the most reliable way to design new potent active molecules having similar scaffolds by utilizing their biological data in computational predictions. In this study, the selected pharmacophore including one hydrogen bond acceptor (F1: Acc& ML), one hydrogen bond donor (F2: Don, Acc& ML) and one hydrophobic feature with an aromatic center (F3: ML/Hyd/Aro) (Fig. 1a) was mapped over active compounds (Fig. 1b). The mapping was evaluated on the basis of their lowest RMSD between query and matching annotations (Fig. 1c, d).

The lowest RMSD indicates better compound fitness to the selected model. Results in Table 2 showed that all the active compounds were able to satisfy the pharmacophoric features of the generated model with RMSD values ranging from 0.3907 to 0.6571 Å along with their most suitable alignment of each compound over query. These results indicated the critical role of aromatic ring substitution which greatly effects the spatial orientation of cyclohexane ring with respect to the pyrazole moiety. This might be the best explanation to understand the differences in their respective antimicrobial activity profile.

Docking simulation to predict the mode of inhibition
After SAR profiling, docking studies were carried out to predict the most suitable binding pose and inhibition mechanism of newly synthesized derivatives. But before docking, based on the principle that similar Compounds tend to bind to the same proteins, we predicted few protein targets reported against reference compounds (ciprofloxacin and fluconazole) and docked our active compounds against them. Binding DB brought in seven different target proteins i.e. Dihydrofolate Reductase (DHFR) (PDB ID 4HOF), Secreted Aspartic Protease...
Barakat et al. Chemistry Central Journal (2018) 12:29

Table 1 Results of cup-plate method expressed as minimum inhibitory concentrations (MIC) of the compounds in (µg/L)

| Entry | Compounds | Gram positive bacteria | Yeast |
|-------|-----------|------------------------|-------|
|       |           | S. aureus ATCC 29213    | C. albicans ATCC 2091 |
|       |           | CPM (mm) | MIC (µg/L) | CPM (mm) | MIC (µg/L) |
| 1     | 4a        | 13       | 32         | 14       | 32         | 14       | 32 |
| 2     | 4b        | 15       | 32         | 13       | 16         | 15       | 16 |
| 3     | 4c        | 13       | 32         | 24       | 32         | 16       | 32 |
| 4     | 4d        | 16       | 32         | 16       | 32         | 18       | 32 |
| 5     | 4e        | 19       | 16         | 15       | 32         | 14       | 64 |
| 6     | 4f        | 14       | 32         | 13       | 64         | 15       | 32 |
| 7     | 4g        | 14       | 32         | 15       | 32         | 16       | 32 |
| 8     | 4h        | 12       | 64         | 14       | 32         | 16       | 32 |
| 9     | 4i        | 14       | 32         | 12       | 64         | 17       | 32 |
| 10    | 4j        | 10       | 64         | 13       | 32         | 10       | 32 |
| 11    | 4k        | 13       | 32         | 13       | 32         | 20       | 8  |
| 12    | 4l        | 16       | 16         | 16       | 32         | 16       | 32 |
| 13    | 4m        | 15       | 32         | 13       | 32         | 12       | 32 |
| 14    | 4n        | 14       | 32         | 13       | 32         | 15       | 32 |
| 15    | 4o        | 13       | 32         | 20       | 32         | 15       | 16 |
| STD   | Ciprofloxacin | ≤ 0.25 | 24 | ≤ 0.25 | 25 | ≤ 0.25 | ND | ND |
|       | Fluconazole | ND | ND | ND | ND | ND | 28 | 0.5 |

(See page 5 of 13 for more content)
hotspot residues Phe117, Tyr225 and Tyr 354. Simultaneously, several hydrophobic interactions were also noticed among compound 4o and the crucial residues i.e. Tyr107, Phe 117, Tyr119, Tyr225, Tyr335. These results predicted TMK (S. aureus) and NMT (C. albicans) as the most probable targets for the antibacterial and antifungal activity of these newly synthesized agents.

**Table 2** RMSD values along with their suitable alignment for Hit Compounds

| Comp. no. | 4b   | 4c   | 4d   | 4e   | 4h   | 4k   | 4l   | 4m   | 4o   |
|-----------|------|------|------|------|------|------|------|------|------|
| RMSD (Å)  | 0.3907 | 0.4715 | 0.4639 | 0.4663 | 0.4662 | 0.5938 | 0.5070 | 0.6571 | 0.5660 |

**Conclusions**

By using one-pot green protocol a series of pyrazole-dimedone derivatives (4a–o) were synthesized in high yields with a broad substrate scope under mild reaction conditions in water mediated by NHEt₂. The requisite compounds were evaluated for their antibacterial and antifungal activities. After experimental investigations,
structure–activity relationship profiling was predicted by ligand-based pharmacophore modeling highlighting three features as a requirement for their antimicrobial activity. While Molecular docking predicted the molecular mechanisms of these derivatives with seven different target proteins. Among them, TMK from *S. aureus* and NMT protein from *C. albicans* were predicted as the most suitable targets for the antibacterial and antifungal activities of these newly synthesized derivatives.

**Experimental**

**Materials and methods**

**General**

“All the chemicals were purchased from Aldrich, Sigma-Aldrich, Fluka etc., and were used without further purification, unless otherwise stated. All melting points were measured on a Gallenkamp melting point apparatus in open glass capillaries and are uncorrected. IR Spectra were measured as KBr pellets on a Nicolet 6700 FT-IR spectrophotometer. The NMR spectra were recorded on a Varian Mercury Jeol-400 NMR spectrometer. $^1$H-NMR (400 MHz), and $^{13}$C-NMR (100 MHz) were run in either deuterated dimethyl sulphoxide (DMSO-$d_6$) or deuterated chloroform (CDCl$_3$). Chemical shifts ($\delta$) are referred in terms of ppm and $J$-coupling constants are given in Hz. Mass spectra were recorded on a Jeol of JMS-600 H. Elemental analysis was carried out on Elmer 2400 Elemental Analyzer; CHN mode”.

**General procedure for Knoevenagel condensation Michael addition for the synthesis of 4a–o (GP1)**

A mixture of aldehyde 1 (1.5 mmol), 5,5-dimethylcyclohexane-1,3-dione 2, (1.5 mmol), 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1.5 mmol) and Et$_2$NH (1.5 mmol, 155 μL) in 3 mL of degassed H$_2$O was stirred at room temperature for 1–12 h until TLC showed complete disappearance of the reactants. The precipitate was removed by filtration and washed with ether ($3 \times 20$ mL). Solid was dried to afford pure products 4a–o.
5-((2,4-Dichlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate diethylaminium salt 4a 4a was prepared according to the general procedure (GP1) from 2,4-dichlorobenzaldehyde yielding orange powdered materials. m.p: 144 °C; IR (CsI cm⁻¹): 3451, 2984, 2868, 2719, 2492, 1598, 1501, 1468, 1380, 1262;    ¹H-NMR (400 MHz, DMSO-d₆): δ 8.08 (d, 1H, J = 7.3 Hz, Ph), 7.93 (d, H, J = 7.3 Hz, Ph), 7.42 (s, 1H, Ph), 7.32–7.04 (m, 5H, Ph), 4.96 (s, 1H, CH=CH), 2.85 (q, 4H, J = 7.3 Hz, CH₂CH₃), 1.11 (t, 6H, J = 7.3 Hz, CH₂CH₃);    ¹³C-NMR (100 MHz, DMSO-d₆): δ = 157.6, 145.5, 142.4, 140.6, 132.1, 131.9, 128.3, 128.0, 126.6, 123.0, 119.1, 100.9, 41.7, 30.9, 13.2, 11.0; LC/MS (ESI): 330.07 [M]+ for C₁₈H₁₆Cl₂N₂; Anal. for C₂₁H₂₄Cl₂N₃O; calcd C, 62.23; H, 5.97; Cl, 17.49; N, 10.37; Found: C, 62.23; H, 5.97; Cl, 17.49; N, 10.37.

3-Hydroxy-2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(phenyl)methyl)-5,5-dimethylcyclohex-2-enone diethylaminium salt 4b 4b was prepared according to the general procedure (GP1) from benzaldehyde yielding orange powdered materials. m.p: 102 °C; IR (CsI cm⁻¹): 3448, 3058, 2957, 2732, 2507, 1582, 1579, 1501, 1454, 1365, 1263;    ¹H-NMR (400 MHz, DMSO-d₆): δ 15.30 (s, 1H, OH), 15.30 (s, 1H, OH), 7.92 (m, 3H, Ph), 7.33–7.07 (m, 7H, Ph), 5.75 (s, 1H, benzyl-H), 2.86 (q, 4H, J = 7.3 Hz, CH₂CH₃), 2.16 (s, 3H, CH₃), 2.12 (t, 6H, J = 7.3 Hz, CH₂CH₃), 1.11 (t, 6H, J = 7.3 Hz, CH₂CH₃), 1.10 (s, 3H, CH₃), 1.00 (s, 3H, CH₃); ¹³C-NMR (100 MHz, DMSO-d₆): δ = 189.8, 157.2, 146.4, 145.8, 145.5, 140.5, 128.4, 128.3, 127.2, 119.1, 102.2, 79.2, 41.4, 30.2, 28.8, 12.9, 12.7, 11.00; LC/MS (ESI): 262.1M]+ for C₁₈H₁₈N₂; Anal. for C₂₉H₃₈N₃O₃; calcd C, 73.08; H, 8.04; N, 8.82; Found: C, 73.07; H, 8.05; N, 8.83.

Diethylammonium 5-((4-chlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate 4c 4c was prepared according to the general procedure (GP1) from 4-chlorobenzaldehyde yielding orange powdered materials. m.p: 92 °C; IR (CsI cm⁻¹): 3450, 2958, 2868, 2732, 2506, 1702, 1579, 1501, 1487, 1387, 1366, 1318, 1263;    ¹H-NMR (400 MHz, DMSO-d₆): δ 15.30 (s, 1H, OH), 7.34–7.07 (m, 7H, Ph),
5.57 (s, 1H, benzyl-H), 2.91 (q, 4H, J = 7.3 Hz, CH₂CH₃), 2.19 (s, 3H, CH₃), 2.12 (s, 2H, CH₂), 0.99 (t, 6H, J = 7.3 Hz, CH₂CH₃), 1.14 (s, 3H, CH₃), 1.15 (s, 3H, CH₃); ¹³C-NMR (100 MHz, DMSO-d₆): \( \delta = 189.8, 157.2, 146.4, 145.8, 145.5, 140.5, 128.4, 128.3, 127.7, 127.2, 119.1, 102.2, 79.2, 41.4, 30.2, 28.8, 12.9, 12.7, 11.00; LC/MS (ESI): 262.1 [M⁺] for C₁₈H₁₇ClN₂; Anal. for C₂₉H₃₆ClN₃O₃; Calcd: C, 73.08; H, 8.04; N, 8.82; Found: C, 73.07; H, 8.05; N, 8.83, Cl, 6.21.

3-Hydroxy-2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(p-tolyl)methyl)-5,5-dimethylcyclohex-2-enone diethylaminium salt 4d 4d was prepared according to the general procedure (GP1) from p-tolualdehyde yielding orange powdered materials. m.p: 104 °C; IR (CsI, cm⁻¹): 3450, 3017, 2956, 2732, 2506, 1683, 1581, 1501, 1455, 1386, 1318, 1260; ¹H-NMR (400 MHz, CDCl₃): \( \delta = 189.8, 168.5, 157.9, 145.9, 140.4, 128.8, 128.7, 128.5, 127.6, 127.3, 121.7, 121.3, 80.3, 41.7, 31.5, 20.9, 12.6, 11.5; LC/MS (ESI): 276.1 [M⁺] for C₁₉H₂₀N₂; Anal. for C₂₉H₃₆ClN₃O₃; Calcld C, 73.08; H, 8.04; N, 8.82; Found: C, 73.07; H, 8.05; N, 8.83.

3-Hydroxy-2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(m-tolyl)methyl)-5,5-dimethylcyclohex-2-enone diethylaminium salt 4e 4e was prepared according to the general procedure (GP1) from m-tolualdehyde yielding orange powdered materials. m.p: 97 °C; IR (CsI, cm⁻¹): 3449, 3033, 2956, 2731, 2506, 1581, 1501, 1387, 1318, 1261; ¹H-NMR (400 MHz, CDCl₃): \( \delta = 189.8, 168.5, 157.9, 145.9, 140.4, 128.8, 128.7, 128.5, 127.6, 127.3, 121.7, 121.3, 80.3, 41.7, 31.5, 20.9, 12.6, 11.5; LC/MS (ESI): 276.1 [M⁺] for C₁₉H₂₀N₂; Anal. for C₂₉H₃₆ClN₃O₃; Calcld C, 73.44; H, 8.22; N, 8.57; Found: C, 73.43; H, 8.23; N, 8.57.
2-((4-Bromophenyl)(5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl)-3-hydroxy-5,5-dimethylcyclohex-2-enone diethylaminium salt 4f

4f was prepared according to the general procedure (GP1) from p-bromobenzaldehyde yielding orange powdered materials.

m.p. 86 °C; IR (KBr, cm⁻¹): 3447, 2957, 2868, 2731, 2500, 1699, 1579, 1501, 1483, 1388, 1263; ¹H-NMR (400 MHz, DMSO-d₆): δ 15.45 (s, 1H, OH), 7.91 (dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.35–7.26 (m, 5H, Ph), 5.50 (s, 1H, benzyl-H), 2.90 (q, 4H, J = 7.3 Hz, CH₂CH₃), 2.13 (s, 3H, CH₃), 2.07 (s, 2H, CH₂), 2.05 (s, 2H, CH₂), 1.14 (t, 6H, J = 7.3 Hz, CH₂CH₃), 1.12 (s, 3H, CH₃), 0.96 (s, 3H, CH₃); ¹³C-NMR (100 MHz, DMSO-d₆): δ = 189.8, 157.2, 155.9, 147.0, 145.8, 145.5, 140.7, 130.4, 129.6, 129.5, 128.4, 128.2, 122.9, 119.0, 118.8, 101.7, 79.7, 41.4, 31.9, 30.1, 28.3, 12.9, 12.8, 11.0; LC/MS (ESI): 340.1 [M⁺] for C₁₄H₁₂BrN₂O₂. Anal. for C₁₄H₁₂BrN₂O₂: calcd C, 41.7, 31.5, 20.9, 12.6, 11.5; Found: C, 73.44, H, 8.22, N, 8.56; Found: C, 73.43, H, 8.23, N, 8.57.

3-Hydroxy-2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(3-nitrophenyl)methyl)-5,5-dimethylcyclohex-2-enone diethylaminium salt 4i

4i was prepared according to the general procedure (GP1) from m-nitrobenzaldehyde yielding white powdered materials.

m.p. 99 °C; IR (CsI, cm⁻¹): 3447, 3067, 2958, 2731, 2560, 1705, 1597, 1502, 1387, 1348, 1265; ¹H-NMR (400 MHz, CDCl₃): δ 15.30 (s, 1H, OH), 8.02 (dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.61 (dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.31–7.19 (m, 5H, Ph), 5.72 (s, 1H, benzyl-H), 2.70 (q, 4H, J = 7.3 Hz, CH₂CH₃), 2.27 (s, 3H, CH₃), 2.24 (s, 2H, CH₂), 2.19 (s, 2H, CH₂), 1.07 (s, 6H, CH₃), 1.02 (t, 6H, J = 7.3 Hz, CH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ = 189.8, 157.9, 145.9, 140.4, 128.7, 128.6, 128.2, 127.9, 127.7, 125.3, 124.8, 121.6, 120.3, 42.3, 31.6, 21.7, 11.4; LC/MS (ESI): 307.1 [M⁺] for C₁₉H₁₇N₂O₃; Anal. for C₁₉H₁₇N₂O₃: calcd C, 66.77; H, 7.15; N, 10.74; Found: C, 66.75; H, 7.16; N, 10.75.

3-Hydroxy-2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(4-methoxyphenyl)methyl)-5,5-dimethylcyclohex-2-enone diethylaminium salt 4j

4j was prepared according to the general procedure (GP1) from anisaldehyde yielding deep orange materials.

m.p. 84 °C; IR (CsI, cm⁻¹): 3451, 2956, 2835, 2732, 2507, 1681, 1598, 1502, 1456, 1366, 1318, 1261; ¹H-NMR (400 MHz, CDCl₃): δ 15.35 (s, 1H, OH), 7.64 (dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.27 (dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.14–6.68 (m, 5H, Ph), 5.59 (s, 1H, benzyl-H), 3.69 (s, 3H, OCH₃), 2.33 (q, 4H, J = 7.3 Hz, CH₂CH₂), 2.27 (s, 3H, CH₃), 2.25 (s, 2H, CH₂), 2.17 (s, 2H, CH₂), 0.99 (s, 6H, CH₃), 0.83 (t, 6H, J = 7.3 Hz, CH₂CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ = 189.8, 157.9, 145.9, 140.4, 136.8, 128.8, 126.5, 124.7, 121.3, 114.4, 113.4, 113.2, 80.3, 55.4, 41.7, 31.4, 11.2; LC/MS (ESI): 292.1 [M⁺] for C₁₉H₁₄NO₂; Anal. for C₁₉H₁₄NO₂: calcd C, 71.12; H, 7.96; N, 8.29; Found: C, 71.11; H, 7.97; N, 8.31.

3-Hydroxy-2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(4-nitrophenyl)methyl)-5,5-dimethylcyclohex-2-enone diethylaminium salt 4h

4h was prepared according to the general procedure (GP1) from p-nitrobenzaldehyde yielding orange powdered materials.

m.p. 99 °C; IR (KBr, cm⁻¹): 3450, 335, 2958, 2869, 2731, 2507, 1598, 1580, 1501, 1387, 1262; ¹H-NMR
(400 MHz, DMSO-<sup>d6</sup>): δ 15.45 (s, 1H, OH), 7.89–7.83 (dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.32–7.28(dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.20–6.94 (m, 5H, Ph), 5.53 (s, 1H, benzyl-H), 2.90 (q, 4H, J = 7.3 Hz, CH2CH3), 2.16 (s, 3H, CH3), 2.11 (s, 2H, CH2), 2.07 (s, 2H, CH2), 1.14 (t, 6H, J = 7.3 Hz, CH2CH3), 1.11 (s, 3H, CH3).<sup>13</sup>C-NMR (100 MHz, DMSO-<sup>d6</sup>): δ = 192.3, 156.1, 146.7, 139.3, 128.7, 128.7, 127.8, 126, 121.7, 121.30, 103.6, 78.8, 42.1, 31.3, 12.6; LC/MS (ESI): 312.0 [M]+ for C<sub>22</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>; Anal. for C<sub>22</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>; calcld C, 67.20; H, 7.52; N, 8.73.

3-Hydroxy-2-((5-hydroxy-3-methyl-1-phenyl-1H-pyrazol-4-yl)(4-trifluoromethylphenyl)methyl)-5,5-dimethylcyclohex-2-enone diethylaminium salt 4l 4l was prepared according to the general procedure (GP1) from p-trifluoromethylbenzaldehyde yielding yellow powdered materials. m.p: 96 °C; IR (CsI, cm<sup>-1</sup>): 3451, 2959, 2870, 2733, 2506, 1615, 1598, 1502, 1387, 1325, 1266; <sup>1</sup>H-NMR (400 MHz, DMSO-<sup>d6</sup>): δ 16.145 (s, 1H, OH), 7.94–7.90 (dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.57–7.44 (dd, 2H, J = 7.3 Hz, 1.5 Hz, Ph), 7.34–7.06 (m, 5H, Ph), 5.76 (s, 1H, benzyl-H), 2.91 (q, 4H, J = 7.3 Hz, CH2CH3), 2.19 (s, 3H, CH3), 2.12 (s, 2H, CH2), 2.10 (s, 2H, CH2), 1.15 (t, 6H, J = 7.3 Hz, CH2CH3), 1.11 (s, 3H, CH3), 1.00 (s, 3H, CH3). <sup>13</sup>C-NMR (100 MHz, DMSO-<sup>d6</sup>): δ = 157.2, 147.0, 145.7, 140.2, 128.6, 128.5, 128.3, 123.3, 119.2, 118.9, 113.6, 102.4, 102.3, 79.2, 41.4, 31.3, 30.1, 28.7, 12.8, 12.6, 11.0; LC/MS (ESI): 330.13 [M]+ for C<sub>18</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>2</sub>; Anal. for C<sub>18</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>2</sub>O; calcld C, 66.17; H, 6.86; F; 10.45; N, 7.71.

5-((2,6-Dichlorophenyl)(2-hydroxy-4,4-dimethyl-6-oxocyclohex-1-en-1-yl)methyl)-3-methyl-1-phenyl-1H-pyrazol-4-olate diethylaminium salt 4m 4m was prepared according to the general procedure (GP1) from 2,6-dichlorobenzaldehyde yielding deep orange powdered materials. m.p: 142 °C; IR (CsI, cm<sup>-1</sup>): 3459, 3117, 3061, 2973, 2834, 2479, 1657, 1646, 1596, 1500, 1431, 1311, 153; <sup>1</sup>H-NMR (400 MHz, DMSO-d6): 8.08 (d, 1H, J = 7.3 Hz, Ph), 7.93 (d, H, J = 7.3 Hz, Ph), 7.42 (s, 1H, Ph), 7.32–7.04 (m, 5H, Ph), 4.96 (s, 1H, CH = C), 2.85 (q, 2H, J = 7.3 Hz, CH2CH3), 2.12 (s, 3H, CH3), 1.11 (t, 6H, J = 7.3 Hz, CH2CH3). <sup>13</sup>C-NMR (100 MHz, DMSO-<sup>d6</sup>): δ = 161.6, 160.1, 150.0, 148.0, 132.9, 132.7, 131.3, 129.0, 128.9, 128.5, 128.1, 118.1, 117.8, 14.4; LC/MS (ESI): 330.07 [M]+ for C<sub>18</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>2</sub>; Anal. for C<sub>18</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>2</sub>O; calcld C, 61.65; H, 3.65; Cl, 21.41; N, 8.46; Found: C, 61.64; H, 3.63; Cl, 21.40; N, 8.44.

Antibacterial activity studies
The antimicrobial studies were carried out according to reported methodology in the following literature reported by Barakat et al. [12, 13, 23] including initial screening and determination of MIC.

In-silico predictions
Pharmacophore modeling
A ligand-based pharmacophore model was developed by using MOE 2017 [24] suite. Where, a training set representing the most active lead analogs [12, 13] was selected, energy minimized and submitted to flexible alignment for analyzing the shared spatial arrangement of their pharmacophoric features. Generated hypotheses were ranked based on their accuracy scoring and atomic alignment for the highest ranked hypotheses, the best pharmacophore showing 100% accuracy was selected. This selected model was validated for its predictive efficacy by overlapping representative active analogs over it and calculating the RMSD (root mean square distance) between the query and mapped compounds.
**Docking simulation**

To predict the most suitable targets and inhibition mechanisms for the antibacterial and antifungal activities of the newly synthesized pyrazole-dimedone derivatives, reference compounds i.e. ciprofloxacin and fluconazole were submitted in Binding DB [25]. Binding DB works on the principle that similar compounds tend to have the same target proteins and seven proteins were chosen; four proteins i.e. Dihydrofolate Reductase (PDB ID 3FYV), Gyrase B (PDB ID 4URM), Thymidylate Kinase (TMK) (PDB ID 4QGG) and Sortase A (PDB ID 2MLM) from *S. aureus* for antibacterial (ciprofloxacin) and three proteins (Dihydrofolate Reductase (DHFR) (PDB ID 4HOF), Secreted Aspartic Protease (PDB ID 3Q70), and N-myristoyl transferase (PDB ID 1YL)) from *C. Albicans* for antifungal (fluconazole) compounds. The crystal structures of the seven target proteins were fetched from Protein Data Bank (www.rcsb.org/pdb) and all the proteins were prepared, charged, protonated and minimized via MOE 2016 suite. The chemical structures of synthesized compounds were built and saved in their 3D conformations by Builder tool incorporated in MOE 2016. Further protonation, minimization, charge application and atom-type corrections were also done by MOE 2016. Before docking, the efficiency of docking software was validated via redocking the crystallized ligand back into the pocket of significant antibacterial and antifungal target proteins. After redocking experiment (Additional file 1: Figures S1 and S2), we found MOE as the appropriate software to continue our in silico work with this software.

**Additional file**

Additional file 1. Additional information.

**Authors’ contributions**

AB conceived and designed the experiments; BMA-Q and MA performed the experiments; AMA analyzed the data; AB contributed reagents/materials/analysis tools; MHA carried out the antimicrobial activity; MT, SN, and ZU‑H carried out pharmacophore modeling and molecular docking studies; AB wrote the analysis tools; MHA carried out the antimicrobial activity; MT, SN, and ZU‑H carried out the pharmacophore modeling and molecular docking studies; AB wrote the paper. All authors read and approved the final manuscript.

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**Competing interests**

The authors declare that they have no competing interests.

**Ethics approval and consent to participate**

Not applicable.

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