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Evaluation of in vivo wound-healing potential of 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone derivatives

K. Vinaya [a], H. Raja Naika [b], C.S. Ananda Kumar [a], S.B. Benaka Prasad [a], S. Chandrappa [a], S.R. Ranganatha [a], V. Krishna [b], K.S. Rangappa [a,*]

[a] Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India
[b] Phytochemistry and Pharmacology Laboratory, Post Graduate Department of Studies and Research in Biotechnology and Bioinformatics, School of Biological Sciences, Kuvempu University, Shankaraghatta 577 451, India

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ABSTRACT

Series of 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone derivatives **9**(**a**–**d**) and **10**(**a**–**d**) were synthesized in good yield. The synthesized compounds were characterized by ^{1}H NMR, LC–MS, FTIR and elemental analysis. All the compounds were screened for *in vivo* wound-healing activity by incision and dead space wound models on Swiss albino rats. Significant wound healing was observed in **10b** and **10d** treated groups as also the epithelialization of the incision wound was faster with a high rate of wound contraction in these groups. The tensile strength of the incision wound was significantly increased in **10b** and **10d** compared to the Nitrofurazone, the standard skin ointment. In dead space wound model also the weight of the granulation was higher indicating increase in collagenation. The SAR correlation studies revealed that the thioamide functional linkage and electron withdrawing groups influence the wound-healing activity.

1. Introduction

Wound infections are common in developing countries because of poor hygienic conditions. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeroginosa*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae* are some important organisms causing wound infection [1]. A wide range of antibiotics is being used at present for treating wound infections, but they are now proved to have adverse effects on the human body system beside, these pathogens develop resistance to the antibiotics targeted against them. In view of this, much attention has been paid to synthesis of biologically active compounds.

Wound healing is a complex multifaceted process that results in the contraction and closure of the wound and restoration of a functional barrier [2]. It is a process of repair that follows injury caused either to the skin or to other soft tissues of the body. Following injury, an inflammatory response starts and the cells below the dermis (the deepest skin layer) begin to increased collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) gets regenerated [3,4]. There are three stages in the process of wound healing: inflammation, proliferation, and remodeling.

Interventions that promote the healing of skin wounds have been in use around millennia. The phrase 'to lick ones wounds' might have been grounded in science to show that mammalian saliva contains some anti-bacterial agents and growth factors that aid in wound resolution [5]. Given this historical background, it is surprising that the modern clinical approach to improving the healed appearance of skin wounds remains largely devoid of drug-based therapies. There are no licensed therapeutics in clinical use that have proven consistency in ameliorating excess deposition of scar tissue – a frequent undesirable resultant of both surgical and non-surgical injuries caused to the skin.

Heterocyclic compounds carrying piperidine skeleton are attractive targets of organic synthesis owing to their pharmacological activities and their wide occurrence in nature [6–8]. Piperidine heterocycles play an important role in the field of medicinal chemistry. Several derivatives of this class such as herbicidal, insecticidal, fungicidal, bactericidal, anti-inflammatory, antihistaminic, hypotensive, anticancer, CNS stimulant and depressant and nerve activities have been found to possess useful biological activities [9–16]. The great importance of benzophenones is fundamentally due to the diverse biological [17] and chemical [18] properties that they possess. The proficiency of benzophenone analogues as chemotherapeutic agent especially as anti-inflammatory is well documented [19].

* Corresponding author. Tel.: +91 821 2419661; fax: +91 821 2412191.
E-mail addresses: rangappaks@gmail.com, rangappaks@chemistry.uni-mysore.ac.in (K.S. Rangappa).

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Benzophenone analogues synthesized by several scientists have been reported as effective *antiinflammatory* agents [20–22].

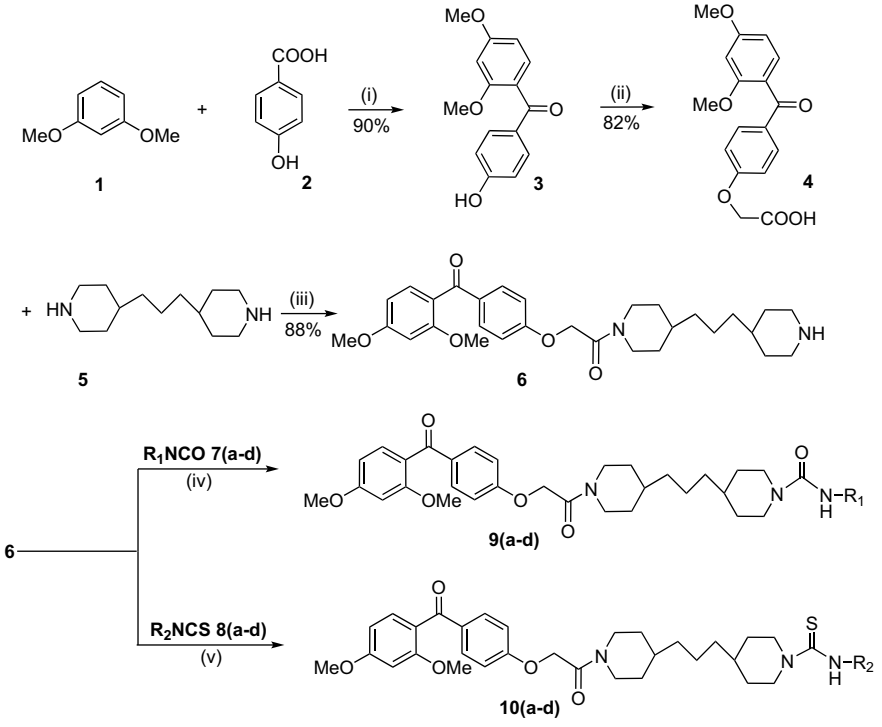
An essential component of the search for new leads in a drug designing program is the synthesis of molecules, which are novel yet resemble known biologically active molecules by virtue of the presence of some critical structural features. Certain small heterocyclic molecules act as highly functionalized scaffolds and are known pharmacophores of a number of biologically active and medicinally useful molecules [23,24]. Anti-inflammatory drugs are widely used in the treatment of various inflammatory skin disorders [25,26]. The rate of wound healing is invariably brought down by these agents [27,28]. In view of the above, we planned to synthesize a system which combines these two biolabile components together to give a compact structure like title compounds and investigate their wound-healing capacity by incision and dead space wound models.

2. Chemistry

For the synthesis of the target key intermediate compound **6**, the reaction sequences outlined in Scheme 1 were followed. (4-Hydroxyphenyl)(2,4-dimethoxyphenyl)methanone (**3**) was synthesized by Friedel–Crafts reaction with 1,3-dimethoxy benzene (**1**) (1.0 eq) and *p*-hydroxy benzoic acid (**2**) (1.2 eq) in the presence of phosphorus oxychloride (7.0 eq) and zinc chloride (2.5 eq) at 60–70 °C for 2 h. The absence of –COOH and presence of phenolic proton peak in ^{1}H NMR and IR spectra confirmed the formation of compound **3**. Treatment of (4-hydroxyphenyl)(2,4-dimethoxyphenyl) methanone (**3**) with chloroacetic acid (4.5 eq) in potassium carbonate (7.0 eq) solution, and refluxed for 10 h gave the *O*-alkylated product. The absence of Ar–OH and presence of –COOH proton peak in **4** confirm the formation of the product. [4-(2,4-Dimethoxy-benzoyl)-phenoxy]-acetic acid (**4**) (1.0 eq) and 4-(3-(piperidin-4-yl)propyl)piperidine (**5**) (1.0 eq) in *N*,*N*-dimethyl formamide (DMF) were taken, to which *N*-methylmorpholine (NMP) (3.0 eq) and 10% of *o*-benzotriazol-1-yl-*N*,*N*,*N*^{1},*N*^{1}-tetramethyl uranium tetrafluoroborate (TBTU) catalyst were added, and reaction mixture was stirred for 5 h at room temperature, which gave target key intermediate **6**. The absence of –COOH proton peak and presence of –NH proton peak confirmed the formation of compound **6**. The nucleophilic substitution reaction of 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) with different substituted aromatic isocyanates (R–N═C═O)/isothiocyanates (R–N═C═S) was carried out in the presence of triethylamine and dichloromethane as solvent with a good yield of 74–80%. The absence of –NH and presence of –CO–NH, –CS–NH proton peak in synthesized derivatives **9**(**a**–**d**) and **10**(**a**–**d**) in proton NMR and IR spectra confirmed the identity of the products. It is also confirmed by IR data, for carboxamide series **9**(**a**–**d**) and thioamide series **10**(**a**–**d**), IR data showed stretching frequency at 3350–3360 cm^{-1} for –NH and 1640–1660 cm^{-1} for –C═O group. The chemical structures and yield of all the synthesized compounds are given in Table 1.

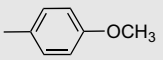
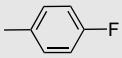
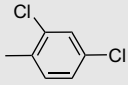
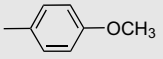
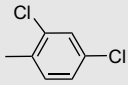
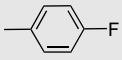
3. Experimental

Infrared (IR) spectra were recorded using a Jasco FTIR-4100 spectrometer in the wave number range of 4000–400 cm^{-1}. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AM 400 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane as an internal standard. The chemical shifts are expressed in δ and the following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet).



Scheme 1. Reaction and reagent conditions: (i) $ZnCl_2$, $POCl_3$, 60–70 °C, 2 h. (ii) $ClCH_2COOH$, K_2CO_3, reflux, 10 h. (iii) *N*-Methylmorpholine, *o*-benzotriazol-1-yl-*N*,*N*,*N*^{1},*N*^{1}-tetramethyl uranium tetrafluoroborate (TBTU), *N*,*N*-dimethyl formamide, 5 h. (iv) Isocyanates **7**(**a**–**d**) (R_1NCO), triethylamine, dichloromethane, 5–6 h. (v) Isothiocyanates **8**(**a**–**d**) (R_2NCS), triethylamine, dichloromethane, 5–6 h. **7a**: 4-methoxyphenyl isocyanate; **8a**: 4-methoxyphenyl isothiocyanate; **7b**: 4-fluorophenyl isocyanate; **8b**: 2-chlorophenyl isothiocyanate; **7c**: 2-chlorophenyl isocyanate; **8c**: 2,4-dichlorophenyl isothiocyanate; **7d**: 2,4-dichlorophenyl isocyanate; **8d**: 4-fluorophenyl isothiocyanate.

Table 1
Chemical structure and yield of the synthesized compounds **9**(**a**–**d**) and **10**(**a**–**d**).

Compound	R_1 and R_2	Yield (%)
9a		78
9b		77
9c		75
9d		80
10a		80
10b		74
10c		78
10d		78

Mass and purity were recorded on a LC-MSD-Trap-XCT. Elemental (CHNS) analyses were obtained on Vario EL III Elementar. The purity of the compounds was checked by thin layer chromatography (TLC). Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck made TLC plates. All the reagents and chemicals were from Sigma Aldrich Chemicals Pvt Ltd.

*3.1. Procedure for the synthesis of (4-hydroxyphenyl) (2,4-dimethoxyphenyl)methanone **3***

A solution of *p*-hydroxy benzoic acid (**2**) (23.99 g, 17.39 mmol) was taken, phosphorus oxychloride (92.9 mL, 101.43 mmol) was added slowly under stirring condition. Then dimethoxybenzene (**1**) (20 g, 14.49 mmol) was added, and finally zinc chloride was added (48.96 g, 36.22 mmol). The reaction mixture was heated at 60–70 °C for 2 h. Progress of the reaction was monitored by TLC. Upon completion, the reaction mass was poured slowly into the ice cold water with stirring. The compound was extracted with ethyl acetate, the organic layer was washed with water and brine solution. Evaporation of the organic layer was carried out under reduced pressure followed by *recrystallisation* by using methanol and water; pure compound with 90% yield was obtained. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.18 (s, 1H, –OH) 7.97 (s, 1H, Ar-H), 7.80 (d, 1H, Ar-H), 7.36 (d, 1H, Ar-H), 7.02 (d, 1H, Ar-H), 6.78 (d, 1H, Ar-H), 3.81(s,3H, – OCH_3), 3.70(s, 3H, – OCH_3) 6.62 (dd, 2H, Ar-H). IR (KBr, cm^{-1}): 1669, 1042, 1258. Anal. Calcd for $C_{15}H_{14}O_4$ (in %): C-69.76, H-5.46. Found C-69.69, H-5.41.

*3.2. Procedure for the synthesis of [4-(2,4-dimethoxy-benzoyl)-phenoxy]-acetic acid **4***

Solutions of (4-hydroxyphenyl)(2,4-dimethoxyphenyl)-methanone (**3**) (15 g, 5.81 mmol), and potassium carbonate (56.21 g, 40.67 mmol) were taken in water (125 mL). Then chloroacetic acid (24.7 g, 26.14 mmol) in portion (1 eq/1 h time gap) was slowly added to the reaction mass and adjusted the pH to 9–10 by adding potassium carbonate solution. The reaction mixture was refluxed for 10 h. Progress of the reaction was monitored by TLC. Upon completion, the reaction mass cooled to the room temperature, diluted with water, acidified using 3 M HCl solution (pH = 4–5) and extracted twice with ethyl acetate. The organic layer was washed with water and brine solution until the bottom impurities were removed. The compound was extracted into the sodium carbonate solution from the organic layer and the aqueous layer was washed by using ethyl acetate to remove remaining initial compound. The aqueous layer was acidified using conc. HCl (pH = 1). The pure compound got precipitated in aqueous solution. The compound was filter and dried. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.75 (s, 1H, –COOH) 7.92 (s, 1H, Ar-H), 7.83 (d, 1H, Ar-H), 7.35 (d, 1H, Ar-H), 7.00 (d, 1H, Ar-H), 6.73 (d, 1H, Ar-H), 6.60 (dd, 2H, Ar-H), 5.18 (s, 2H, –OCH_2), 3.81(s, 3H, – OCH_3), 3.72(s, 3H, – OCH_3). IR (KBr, cm^{-1}): 1674, 1045, 1248. Anal. Calcd for $C_{17}H_{16}O_6$ (in %): C-64.55, H-5.10. Found C-64.50, H-5.04.

*3.3. Procedure for the synthesis of 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6***

A solution of [4-(2,4-dimethoxy-benzoyl)-phenoxy]-acetic acid (**4**) (5 g, 1.57 mmol) in dry *N*,*N*-dimethyl formamide was taken, 4-(3-(piperidin-4-yl)propyl)piperidine **5** (3.32 g, 1.57 mmol) was added to the solution, and then *N*-methylmorpholine (4.78 g, 4.73 mmol) and 10% of the TBTU catalyst were added. The reaction mixture was stirred at room temperature for 5 h and progress of the reaction was monitored by TLC. Upon completion of the reaction, water was added and the reaction mixture was filtered, washed with ether and dried under vacuum. A pink amorphous solid compound with 88% yield was obtained. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.02 (s, 1H, Ar-H), 7.78 (d, 1H, Ar-H), 7.34 (d, 1H, Ar-H), 6.97 (d, 1H, Ar-H), 6.78 (s, 1H, Ar-H), 6.60 (dd, 2H, Ar-H), 5.31 (s, 2H, –OCH_2), 3.84 (s, 3H, –OCH_3), 3.72 (s, 3H, –OCH_3), 2.87–3.03 (m, 8H, –N–CH_2–), 2.05 (s, 1H, –NH), 2.02 (br s, 2H, –CH_2–), 1.22–1.32 (m, 14H, –CH_2–). IR (KBr, cm^{-1}): 1687, 1040, 1249. Anal. Calcd for $C_{30}H_{40}N_2O_5$ (in %): C-70.84, H-7.93, N-5.51. Found C-70.79, H-7.89, N-5.46.

*3.4. General procedure for synthesis of 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone derivatives **9**(**a**–**d**) and **10**(**a**–**d**)*

A solution of 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone (**6**) (1.0 eq) in dry dichloromethane was taken and cooled to 0–5 °C in an ice bath. Triethylamine (3.0 eq) was added to this cold reaction mixture and stirred for 10 min, and then different isocyanates (1.0 eq) or isothiocyanates (1.0 eq) were added, and allowed to stir at room temperature for 5–6 h. Progress of the reaction mixture was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. The organic layer was washed with 10% ammonium chloride solution and finally water wash was given to organic layer and dried with anhydrous sodium sulphate, the solvent was evaporated to get crude product which was purified by

column chromatography over silica gel (60–120 mesh) using hexane:ethyl acetate (8:2) as eluent.

3.4.1. Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxy-benzoyl)phenoxy]acetyl]-4-piperidyl] propyl]-N-(4-methoxyphenyl)piperidine-1-carboxamide ***9a***

The product obtained was pale yellow oily from 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) (0.25 g, 0.491 mmol) and 4-methoxyphenyl isocyanate (**7a**) (0.328 g, 2.27 mmol) and triethylamine (0.667 g, 6.81 mmol). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.23 (s, 1H, –NH), 7.81 (d, 2H, Ar-H), 7.33 (m, 2H, Ar-H), 6.95 (m, 4H, Ar-H), 6.84 (d, 1H, Ar-H), 6.59 (d, 2H, Ar-H), 5.29 (s, 2H, –OCH_2), 3.89 (s, 3H, –OCH_3), 3.83 (s, 3H, –OCH_3), 3.73 (s, 3H, –OCH_3), 2.85–2.98 (m, 8H), 2.05 (br s, 2H), 1.09–1.28 (m, 14H). IR (KBr, cm^{-1}): 3356, 2889, 1716, 1648, 1293, 1250, 1124, 1105. MS (ESI) *m*/*z*: 658.34 (M + H$^+$). Anal. Calcd for $C_{38}H_{47}N_3O_7$ (in %): C-69.38, H-7.20, N-6.39. Found C-69.44, H-7.26, N-6.36.

3.4.2. Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxy-benzoyl)phenoxy]acetyl]-4-piperidyl] propyl]-N-(4-fluorophenyl)piperidine-1-carboxamide ***9b***

The product obtained was pale yellow oily from 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) (0.25 g, 0.491 mmol) and 4-fluorophenyl isocyanate (**7b**) (0.301 g, 2.27 mmol) and triethylamine (0.667 g, 6.81 mmol). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.19 (s, 1H, –CONH), 7.79 (d, 2H, Ar-H), 7.27–7.33 (m, 2H, Ar-H), 6.94 (m, 4H, Ar-H), 6.86 (d, 1H, Ar-H), 6.54 (d, 2H, Ar-H), 5.25 (s, 2H, –OCH_2), 3.87 (s, 3H, –OCH_3), 3.72 (s, 3H, –OCH_3), 2.89–2.98 (m, 8H), 2.81 (br s, 2H), 1.74 (m, 2H), 1.09–1.26 (m, 12H). IR (KBr, cm^{-1}): 3346, 2885, 1710, 1639, 1287, 1252, 1121, 1039. MS (ESI) *m*/*z*: 646.32 (M + H$^+$). Anal. Calcd for $C_{37}H_{44}FN_3O_6$ (in %): C-68.82, H-6.87, N-6.71. Found C-68.88, H-6.82, N-6.73.

3.4.3. Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxy-benzoyl)phenoxy]acetyl]-4-piperidyl] propyl]-N-(2-chlorophenyl)piperidine-1-carboxamide ***9c***

The product obtained was pale yellow oily from 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) (0.25 g, 0.491 mmol) and 2-chlorophenyl isocyanate (**7c**) (0.337 g, 2.27 mmol) and triethylamine (0.667 g, 6.81 mmol). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.20 (s, 1H, –NH), 7.80 (d, 2H, Ar-H), 7.35 (m, 2H, Ar-H), 7.12 (m, 4H, Ar-H), 6.89 (d, 1H, Ar-H), 6.62 (d, 2H, Ar-H), 5.27 (s, 2H, –OCH_2), 3.84 (s, 3H, –OCH_3), 3.72 (s, 3H, –OCH_3), 2.83–3.0 (m, 8H), 2.03 (br s, 2H), 1.12–1.28 (m, 14H). IR (KBr, cm^{-1}): 3361, 2920, 2862, 1728, 1674, 1278, 1256, 1123, 1045, 725. MS (ESI) *m*/*z*: 662.30 (M + H$^+$). Anal. Calcd for $C_{37}H_{44}ClN_3O_6$ (in %): C-67.11, H-6.70, N-6.35. Found C-67.16, H-6.77, N-6.41.

3.4.4. Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxy-benzoyl)phenoxy]acetyl]-4-piperidyl] propyl]-N-(2,4-dichlorophenyl)piperidine-1-carboxamide ***9d***

The product obtained was pale yellow oily from 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) (0.25 g, 0.491 mmol) and 2,4-dichlorophenyl isocyanate (**7d**) (0.413 g, 2.27 mmol) and triethylamine (0.667 g, 6.81 mmol). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.22 (s, 1H, –NH), 7.76 (d, 1H, Ar-H), 7.33 (d, 1H, Ar-H), 7.25 (d, 2H, Ar-H), 7.02 (m, 2H, Ar-H), 6.94 (d, 1H, Ar-H), 6.68 (d, 2H, Ar-H), 6.51 (br s, 1H, Ar-H), 5.31 (s, 2H, –OCH_2), 3.84 (s, 3H, –OCH_3), 3.73 (s, 3H, –OCH_3), 2.87–3.02 (m, 8H), 2.02 (br s, 2H), 1.12–1.22 (m, 14H). IR (KBr, cm^{-1}): 3356, 2935, 2874, 1731, 1640, 1270, 1248, 1120, 1041, 723. MS (ESI) *m*/*z*: 697.25 (M + H$^+$). Anal. Calcd for $C_{37}H_{43}Cl_2N_3O_6$ (in %): C-63.79, H-6.22, N-6.03. Found C-63.84, H-6.18, N-6.09.

3.4.5. Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxy-benzoyl)phenoxy]acetyl]-4-piperidyl] propyl]-N-(4-methoxyphenyl)piperidine-1-carbothioamide ***10a***

The product obtained was pale yellow oily from 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) (0.25 g, 0.491 mmol), 4-methoxyphenyl isothiocyanate (**8a**) (0.292 g, 1.77 mmol) and triethylamine (0.667 g, 6.81 mmol). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.25 (s, 1H, –NH), 7.79 (d, 2H, Ar-H), 7.35 (m, 2H, Ar-H), 6.96 (m, 4H, Ar-H), 6.82 (d, 1H, Ar-H), 6.56 (d, 2H, Ar-H), 5.25 (s, 2H, –OCH_2), 3.85 (s, 3H, –OCH_3), 3.80 (s, 3H, –OCH_3), 3.72 (s, 3H, –OCH_3), 2.02 (br s, 8H), 1.58–1.75 (br s, 4H), 1.09–1.22 (m, 12H), 1.0 (m, 4H). IR (KBr, cm^{-1}): 2939, 2881, 1730, 1643, 1274, 1245, 1128, 1117, 1041. MS (ESI) *m*/*z*: 674.32 (M + H$^+$). Anal. Calcd for $C_{38}H_{47}N_3O_6S$ (in %): C-67.73, H-7.03, N-6.24. Found C-67.80, H-6.98, N-6.28.

3.4.6. Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxy-benzoyl)phenoxy]acetyl]-4-piperidyl] propyl]-N-(2-chlorophenyl)piperidine-1-carbothioamide ***10b***

The product obtained was pale yellow oily from 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) (0.25 g, 0.491 mmol), 2-chlorophenyl isothiocyanate (**8b**) (0.30 g, 1.77 mmol) and triethylamine (0.667 g, 6.81 mmol). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.19 (s, 1H, –NH), 7.81 (d, 2H, Ar-H), 7.33 (m, 2H, Ar-H), 7.21 (m, 4H, Ar-H), 6.91 (d, 1H, Ar-H), 6.60 (d, 2H, Ar-H), 5.29 (s, 2H, –OCH_2), 3.83 (s, 3H, –OCH_3), 3.70 (s, 3H, –OCH_3), 2.85–2.98 (m, 8H), 2.05 (br s, 2H), 1.12–1.2 (m, 14H). IR (KBr, cm^{-1}): 2952, 2876, 1741, 1648, 1270, 1241, 1129, 1097, 722. MS (ESI) *m*/*z*: 678.27 (M + H$^+$). Anal. Calcd for $C_{37}H_{44}ClN_3O_5S$ (in %): C-65.52, H-6.54, N-6.20. Found C-65.58, H-6.59, N-6.26.

3.4.7. Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxy-benzoyl)phenoxy]acetyl]-4-piperidyl] propyl]-N-(2,4-dichlorophenyl)piperidine-1-carbothioamide ***10c***

The product obtained was pale yellow oily from 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) (0.25 g, 0.491 mmol), 2,4-dichlorophenyl isothiocyanate (**8c**) (0.361 g, 1.77 mmol) and triethylamine (0.667 g, 6.81 mmol). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.17 (s, 1H, –NH), 7.75 (d, 1H, Ar-H), 7.35 (d, 1H, Ar-H), 7.25 (d, 2H, Ar-H), 7.05 (m, 2H, Ar-H), 6.95 (d, 1H, Ar-H), 6.70 (d, 2H, Ar-H), 6.54 (br s, 1H, Ar-H), 5.34 (s, 2H, –OCH_2), 3.82 (s, 3H, –OCH_3), 3.73 (s, 3H, –OCH_3), 2.85–2.98 (m, 8H), 2.05 (br s, 2H), 1.09–1.22 (m, 14H), 1.0 (m, 4H). IR (KBr, cm^{-1}): 2959, 2880, 1735, 1653, 1276, 1240, 1124, 1058, 727. MS (ESI) *m*/*z*: 713.23 (M + H$^+$). Anal. Calcd for $C_{37}H_{43}Cl_2N_3O_5S$ (in %): C-62.35, H-6.08, N-5.90. Found C-62.41, H-6.13, N-5.96.

3.4.8. Synthesis of 4-[3-[1-[2-[4-(2,4-dimethoxy-benzoyl)phenoxy]acetyl]-4-piperidyl] propyl]-N-(4-fluorophenyl)piperidine-1-carbothioamide ***10d***

The product obtained was pale yellow oily from 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl) -piperidin-1-yl]-ethanone (**6**) (0.25 g, 0.491 mmol), 4-fluorophenyl isothiocyanate (**8d**) (0.271 g, 1.77 mmol) and triethylamine (0.667 g, 6.81 mmol). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 9.23 (s, 1H, –NH), 7.80 (d, 2H, Ar-H), 7.28 (m, 2H, Ar-H), 6.96 (m, 4H, Ar-H), 6.53 (d, 2H, Ar-H), 5.27 (s, 2H, –OCH_2), 3.92 (s, 3H, –OCH_3), 3.83 (s, 3H, –OCH_3), 2.85–2.92 (m, 8H), 2.08 (br s, 2H), 1.09–1.19 (m, 14H), 1.0 (m, 4H). IR (KBr, cm^{-1}): 2892, 1717, 1632, 1280, 1252, 1125, 1041. MS (ESI) *m*/*z*: 662.30 (M + H$^+$). Anal. Calcd for $C_{37}H_{44}FN_3O_5S$ (in %): C-67.15, H-6.70, N-6.35. Found C-67.21, H-6.65, N-6.36.

3.5. Pharmacological activity

3.5.1. Drug formulations

Two types of drug formulations were prepared from synthesized compounds. For topical application, 1% w/w ointment with cream base from all test samples was prepared by fusion method (melting ingredients method) as described by Bharath [29]. For each 1000 g of ointment cream base, 20 g white bees wax, 30 g hard paraffin, 50 g cetyl alcohol were added and placed in an evaporating dish. White soft paraffin (900 g) on a butter paper transferred into the dish. The dish was placed on water bath, by lowering the volume in dish as far as possible on the water bath, stirred well for melting the ingredients. The dish was removed from the water bath, the content was decanted and strained into another hot dish to remove foreign matter and stirred until the ointment cooled and it was allowed to settle. For oral administration, suspensions were prepared by dissolving 4 mg/mL of the synthesized compound incorporate 1% (w/v) of Tween-80, which served as a suspension medium for the compounds. The drugs were administered orally through a feeding tube. All formulations were prepared fresh as and when required.

3.5.2. Animals

Male Wistar strain rats of either sex weighing 150–200 g were procured from the Central animal house, National College of Pharmacy, Shimoga, Karnataka. They were maintained at standard housing conditions and fed with commercial diet (Hindustan Lever Ltd., Bangalore) and watered *ad libitum* during the experiment. The Institutional Animal Ethical Committee (Reg. No. 144/1999/CPCSEA/SMG) permitted the study. The staircase method [30] was adopted for the determination of the acute toxicity. Healthy albino mice of either sex weighing 20–25 g were used to determine the safer dose. The drugs were administered orally.

3.5.3. Wound-healing activity

Ten groups of animals containing four each were used for each of the incision and dead space wound models. The animals of group I were considered as the control, the animals of group II served as the reference standard and were treated with 0.2% (w/w) Nitrofurazone ointment (Aventis Pharma Limited, Pune, India). The animals of groups III, IV, V, and VI were treated with compounds **9**(**a**–**d**), and the groups VII, VIII, IX, and X were treated with compounds **10**(**a**–**d**), respectively.

In incision wound model [31], 6 cm long paravertebral incisions were made through full thickness of the skin on either side of the vertebral column of the rat. Care was taken to see that incision was at least 1 cm lateral to vertebral column. The wounds were closed with interrupted sutures of 1 cm apart using a surgical thread (No. 000) and curved needle (No. 11). The wounds were left undressed and drugs were topically applied to the wound once a day, till the completion of healing. The skin breaking strength of the 10-day-old wound was measured by continuous constant water technique of Lee and Tong [32]. The skin breaking strength is expressed as the minimum weight (in g) of water necessary to bring about the gapping of the wound.

For dead space wound model the animals were anaesthetized with light ether anesthesia and the dead space wounds were created by subcutaneous implantation of sterilized cylindrical grass piths (2.5 cm × 0.3 cm), one on either side on the dorsal paravertebral surface of the rats. The granulation tissues formed on the grass piths were removed on the 10th post wounding day and subjected to breaking strength test and histological study. Ten percentage of neutral formalin solution was used to fix the granulation tissues for 24 h and dehydrated with a sequence of ethanol–xylene series of solutions [33]. The inflicted materials were embedded with paraffin at 40–60 °C. Microtome sections of 10 μm thicknesses were taken. The processed sections were stained with hematoxylin–eosin and observed under microscope.

3.5.4. Statistical analysis

The results are expressed as mean ± S.E. of four animals in each group. The data were evaluated by one-way ANOVA followed by Tukey's pair-wise comparison test using statistical software SPSS Inc. (Chicago, USA). The significance at $^{*}P < 0.05$ and $^{**}P < 0.01$ levels were compared with that of control.

4. Results

The promotion of wound-healing activity is also well gauged by the tensile strength of the incision and dead space wound model. Generally wound-healing agents have the property to enhance the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibers [34]. Significant wound-healing activity was observed in animals treated with the compounds **9**(**a**–**d**) and **10**(**a**–**d**) compared with those which received the reference standard and control treatments. In the dead space wound model, the compounds **10b**, **10d** showed high skin-breaking strength up to 623.25 ± 3.48 g and 645.69 ± 4.09 g as compared with the control group of animals. A significant ($P < 0.01$) increase was observed in the weight of the granulation tissue in animals treated with the synthesized compounds. Compounds **9c**, **10c** showed less skin-breaking strength of up to 541.82 ± 4.25 g and 619.15 ± 7.78 g respectively. The other compounds showed poor epithelialization. In the incision

Table 2
Effect of oral administration of compounds **9**(**a**–**d**) and **10**(**a**–**d**) on dead space wound model.

Compound	Granulation tissue		Breaking strength (g)
	Wet weight (mg/100 g)	Dry weight (mg/100 g)	
Control	80.54 ± 2.26	12.36 ± 0.43	355.42 ± 6.44
Nitrofurazone	134.79 ± 1.89**	26.04 ± 2.06**	623.25 ± 3.48**
9a	112.48 ± 5.31**	21.59 ± 3.16*	414.19 ± 2.33**
9b	91.31 ± 4.09	17.80 ± 1.59*	451.60 ± 2.87**
9c	104.85 ± 2.08**	21.01 ± 3.78	541.82 ± 4.25**
9d	79.00 ± 1.28	17.81 ± 0.61**	361.60 ± 4.32
10a	80.54 ± 2.26	12.36 ± 0.43	355.42 ± 6.44
10b	134.79 ± 1.89**	26.04 ± 2.06**	623.25 ± 3.48**
10c	88.02 ± 0.72*	21.02 ± 0.70**	619.15 ± 7.78**
10d	122.94 ± 0.67**	26.27 ± 0.43**	645.69 ± 4.09**

Each value represents mean ± SE of 4 animals.
*$P < 0.05$ compared to control.
**$P < 0.01$ compared to control.

Table 3
Effect of oral administration of compounds **9**(**a**–**d**) and **10**(**a**–**d**) on incision wound model.

Compound	Breaking strength (g)
Control	371.66 ± 10.45
Nitrofurazone	875.00 ± 85.39**
9a	503.89 ± 52.09*
9b	552.06 ± 60.16*
9c	605.49 ± 65.05**
9d	445.12 ± 54.16*
10a	675.00 ± 85.39**
10b	748.38 ± 79.31**
10c	612.50 ± 119.68*
10d	760.88 ± 82.92**

Each value represents mean ± SE of 4 animals.
*$P < 0.05$ compared to control.
**$P < 0.01$ compared to control.

wound model, **10b** and **10d** treated animals demonstrated high skin-breaking strength up to 748.38 ± 79.31 g and 760.88 ± 82.92 g, respectively. A significant ($P < 0.01$) increase was observed in the weight of the granulation tissue in the animals treated with the synthesized compounds. Compounds **9c** and **10c** showed less skin-breaking strength up to 605.49 ± 65.05 g and 612.50 ± 119.68 g respectively.

The data presented in Tables 2 and 3 show that the tensile strength of the incision wound was significantly increased in the animals treated with compounds **10**(**a**–**d**) and it was similar to that observed with the reference drug Nitrofurazone. A moderate gain in tensile strength was observed in compounds **9**(**a**–**d**) treated animals and it was not significant when compared with control groups. The effect of oral administration of suspensions of compounds **9**(**a**–**d**) and compounds **10**(**a**–**d**) on dead space wound model was assessed by the weight of granulation tissue and its tensile strength. Enhanced collagen maturation is indicated by increased cross-linking of collagen fibers. The increase in weight of the granulation tissue is indication of the presence of higher protein content. Among the treated animals the response was best in compounds **10**(**a**–**d**) treated animals.

Histological section of the granuloma tissue of control animal showed incomplete healing with less epithelialization, macrophages and lesser collagen formation indicating incomplete healing of the wound (Fig. 1A). Nitrofurazone applied section of granuloma tissue showed complete epithelialization and development deposition of collagen fibers (Fig. 1B). The section of the granuloma tissue treated with compound **9c** showed moderate epithelialization and collagenation and also retention of macrophages with moderate epithelialization and fibrosis. But the compounds **10b** and **10d** treated animals showed complete epithelialization and increased collagen formation and complete healing with more of fibroblasts (Figs. 2 and 3).

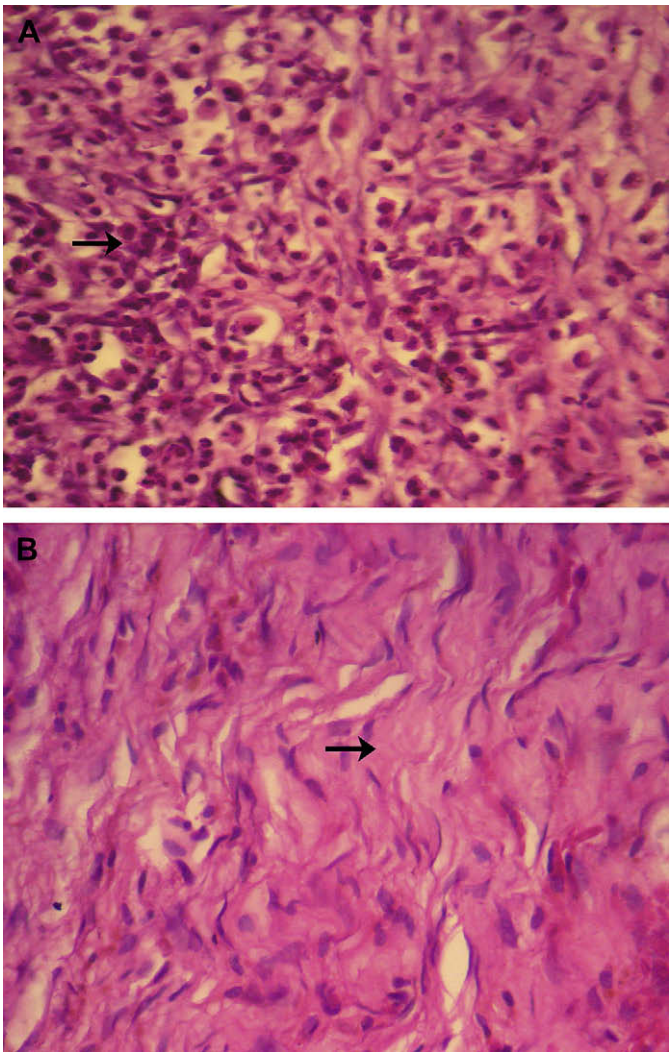


Fig. 1. (A) Histological section of the granuloma tissue of control animal showing incomplete healing with less epithelialization arrow showing macrophage and lesser collagen formation indicated incomplete healing of the wound (H&E, 10×). (B) Histological section of granuloma tissue of standard skin ointment Nitrofurazone applied animal showing complete epithelialization. Arrowhead indicates the deposition of collagen fibers (H&E, 10×).

5. Discussion

Control of inflammation is an important feature of a wound-healing process, since excessive inflammation impedes wound healing [35]. Such prolonged, inflammation and its consequent effect is a major challenge in recalcitrant wounds. The synthesis of 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone amide and thioamide derivatives with different isocyanates/isothiocyanates containing substituted aromatic groups led to the production of novel derivatives **9**(**a**–**d**) and **10**(**a**–**d**). These set of compounds were tried in our study to assess the extent of wound-healing capacity on various phases of wound healing, which run concurrently, but independent of each other. The standard drug Nitrofurazone was used as a reference to assess the healing potency of the proposed drug. The results of the present study clearly indicated that **10b** and **10d** enhanced healing of wounds, while other synthesized compounds failed to show any effect. The activity may be attributed to the anti-inflammatory activity of the piperidine [13] and benzophenone [20–22] moieties and also the presence of electron withdrawing chloro and fluoro groups on the substituted thioamide moiety.

The breaking strength is the strength of a healing wound and is measured experimentally by the amount of force required to disrupt it. In the beginning, a wound will be having little breaking strength because the clot will be holding the edges together. Thereafter, breaking strength increases rapidly as collagen deposition increases and cross linkages are formed between the collagen fibers. Shirwaikar et al. [36] and Singh et al. [37] in their studies also showed significant increase in the breaking strength of wounds in animals treated with synthesized compounds.

The presence of any foreign body in the subcutaneous area initiates the formation of granulation tissue around the wound, having the appearance of pink granules protruding from the floor of

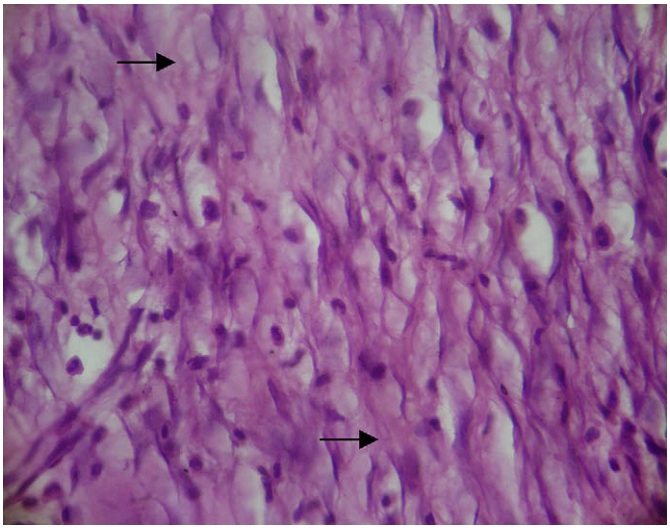


Fig. 2. Histological section of the granuloma tissue from **10b** treated animal removed after 10th day of treatment showing complete epithelialization and increased collagen deposition. Arrows showing increase in collagen formation and complete healing with more of fibroblasts (H&E, 10×).

the wound. When microscopically observed these granules show newly formed capillaries, fibroblasts and leucocytes. As more and more collagen fibers are formed, vascularizatison tissue decreases. The breaking strength of the granulation tissue increases proportionately with the collagen deposition. Due to oral administration of compounds **9**(**a**–**d**) and **10**(**a**–**d**), the breaking strength of the granulation tissue increased. Earlier reports indicate that the increase in weight of the granulation tissue is due to the higher content of protein [38].

Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as close as possible to its normal state. Wound contracture is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage.

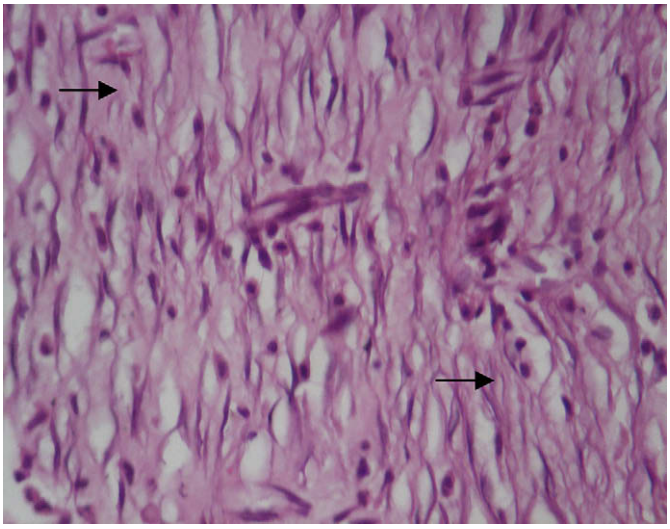


Fig. 3. Histological section of the granuloma tissue from **10d** treated animal removed after 10th day of treatment showing complete epithelialization and increased collagen deposition. Arrows showing increase in collagen formation and complete healing with more of fibroblasts (H&E, 10×).

In the maturation phase, which is the final phase of wound healing the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. Granulation tissue formed in the final part of the proliferative phase is primarily composed of fibroblasts, collagen, edema, and new small blood vessels. The increase in dry granulation tissue weight in the test treated animals suggests higher protein content.

From the results obtained, structure–activity relationship (SAR) can be drawn for compounds **9**(**a**–**d**) and **10**(**a**–**d**). In this connection, several different electron donating or electron withdrawing groups attached to phenyl ring as substituents have been studied for wound-healing efficacy. Upon introduction of electron donating methoxy group in **9a**, **10a** of phenyl ring showed poor wound-healing activity, on the other hand remaining compounds showed superior wound-healing activity with electron withdrawing chloro and fluoro groups. Similarly the SAR study for carboxamide derivatives **9**(**a**–**d**) revealed that, electron withdrawing groups **9b**, **9c** and **9d** attached to phenyl ring as substituents showed superior healing activity compared to the electron donating group **9a**. On the other hand, compounds **9c** and **10b** with one electron withdrawing chloro group at position -2 showed superior activities compared to **9d** and **10c** having two chloro groups at the -2nd and -4th position. Finally *N*-aryl thioamides **10**(**a**–**d**) substituted derivatives showed potent wound-healing activity compared to those having *N*-aryl amides **9**(**a**–**d**). The above four SAR correlation studies revealed that the nature of the functional linkage (amide/thioamide) influences the wound-healing activity. Recent studies with 2-[4-(2,4-dimethoxy-benzoyl)-phenoxy]-1-[4-(3-piperidin-4-yl-propyl)-piperidin-1-yl]-ethanone **6** have shown that substituents like sulfonamides and carboxamides have potent antimicrobial properties [39], may also have a role in wound contraction and increased rate of epithelization.

6. Conclusion

The size and lipophilicity of the substitution on the phenyl moiety are considered to be key factors in determining the wound-healing activity. Halogens like chlorine or fluorine are very useful to modulate the electronic effects on phenyl rings of the synthesized compounds. Moreover, these atoms may also influence the steric characteristics and the hydrophilic–hydrophobic balance of the molecule. On the other hand, ketones, amide and thioamide functional groups have different steric, electronic and lipophilic characteristics. These structural requirements are present in the synthesized compounds and they are expected to get strong wound-healing property of the benzophenones containing a substituted *N*-trimethylene dipiperidine moiety. Therefore, this work presents a novel class of potent, wound-healing activities of the compounds. Among the compounds tested **10b** and **10d** proved to be the most effective.

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