Saudi Pharmaceutical Journal (2016) **24**, 705–717

King Saud University

Saudi Pharmaceutical Journal

www.ksu.edu.sa
www.sciencedirect.com

SHORT COMMUNICATION

Quinoline based furanones and their nitrogen analogues: Docking, synthesis and biological evaluation

Sukhbir Lal Khokra [a], Jyoti [a], Chetan [b], Pawan Kaushik [a], M.M. Alam [c], M.S. Zaman [c], Aftab Ahmad [d], Shah Alam Khan [e], Asif Husain [c,*]

[a] *Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, Haryana, India*
[b] *Department of Microbiology, Kurukshetra University, Kurukshetra, Haryana, India*
[c] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi, India*
[d] *Health Information Technology Department, Jeddah Community College, King Abdulaziz University, Jeddah, Saudi Arabia*
[e] *Department of Pharmacy, Oman Medical College, Muscat, Oman*

Received 19 March 2015; accepted 24 May 2015
Available online 11 June 2015

KEYWORDS

In silico;
Butenolide;
Pyrrolone;
Antimicrobial;
Analgesic;
Anti-inflammatory

Abstract A small library of twenty-four quinoline based butenolides also known as furanones and their nitrogen analogues was prepared by using two different aroylpropionic acids, viz. 3-(2-naphthoyl)propionic acid (**3**) and 3-(biphenyl-4-yl)propionic acid (**4**), as starting materials. The 3-aroylpropionic acids were reacted with different 6-substituted-2-chloroquinolin-3-carbalde hydes (**2a–d**) to obtain the corresponding furan-2(3*H*)-ones (**5a–h**). The purified and characterized furanones were then converted into their corresponding 2(3*H*)-pyrrolones (**6a–h**) and *N*-benzyl-pyrrol-2(3*H*)-ones (**7a–h**). The antimicrobial activities of the title compounds were evaluated against two strains of each Gram +ve (*Staphylococcus aureus* and *Bacillus subtilis*), Gram −ve bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and against fungal strains of *Aspergillus niger* and *Aspergillus flavus*. *In vivo* anti-inflammatory potential of the title compounds was investigated by standard method. Majority of the compounds showed significant antibacterial activity against both the Gram +ve strains. Eight most potent anti-inflammatory compounds (**5b, 5d, 5h, 6b, 7b, 7d, 7f, 7h**) which exhibited >53% inhibition in edema, were also screened for their *in vivo* analgesic activity. All the tested compounds were found to have significant reduction in ulcerogenic action but only three compounds (**5d, 5h** and **7h**) showed comparable analgesic activity to standard drug, diclofenac. The results were also validated using *in silico* approach and maximum mol doc score

* Corresponding author at: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110 062, India. Tel.: +91 11 26059681, 26059688x5647, mobile: +91 989 1116086; fax: +91 11 26059686.
E-mail addresses: drasifhusain@yahoo.com, ahusain@jamiahamdard.ac.in (A. Husain).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

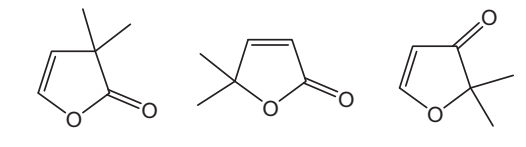
http://dx.doi.org/10.1016/j.jsps.2015.05.002

was obtained for compounds **7a–h**. On comparing the *in vivo* and *in silico anti-inflammatory* results of synthesized compounds, *N*-benzyl pyrrolones (**7a–h**) emerged as the potent anti-inflammatory agents. It was also observed that compounds that possess electron withdrawing group such as —Cl or NO_2 are more biologically active.

1. Introduction

Butenolides, also known as butyrolactones, are five membered heterocyclic compounds occurring naturally in many medicinal plants (Mao et al., 2011). Natural products containing butenolide ring system have been known to exhibit a wide range of useful and significant biological actions. Chemically these are oxidized furans, which are considered as an important scaffold to synthesize compounds of biological and pharmaceutical importance. In recent years, a large number of synthetic compounds containing butenolide nucleus were prepared and studied for various interesting biological actions in search of potent therapeutic agents (Lattmann et al., 2004; Rossi et al., 1998; Hashem et al., 2014). Butenolides consist of four carbon unsaturated γ-lactone ring and occur in numerous phytochemicals in three different forms (Fig. 1) depending upon the relative positions of the carbonyl group and the double bond in the hetero ring such as 2,3-dihydrofuran-2-ones or furan-2(3*H*)-ones, 2,5-dihydrofuran-2-ones or furan-2(5*H*)-ones and 3,2-dihydrofuran-3-ones or furan-3(2*H*)-ones (Allison et al., 1992).

Furanones and their open ring (acyclic) products serve as precursors for the syntheses of large number of physiologically active heterocyclic compounds vis-a-vis can also be fused or combined with other heterocyclic moieties (Allison et al., 1992; Flower, 2003). A number of nitrogen containing heterocyclic systems which exhibit promising biological activities and are prepared from butenolides include pyrrolones, pyridazinones, pyrazoles, isothiazolones, oxadiazoles, triazoles, etc (Bekhit and Abdel-Azeim, 2004; Bailly et al., 2008; Hashem et al., 2014). Several research studies conducted elsewhere have shown that butenolides (furanones) possess wide spectrum of biological activities such as antibacterial, antifungal, antiviral, antioxidant, antimalarial, anticonvulsant, anti-inflammatory, COX-II inhibition, analgesic, antitumor, and anticancer properties (Albrecht et al., 2008; Moosavi-Movahedi et al., 2003; Levy et al., 2003; Lattmann et al., 2004; Hashem et al., 2014). Recently, there has been a great interest in preparing arylidene butenolides, which have a large spectrum of important and potential biological activities (Lattmann et al., 2005; Khan and Husain, 2002; Leite et al., 1999). The γ-lactone ring of butenolides is quite reactive and therefore employed as a building block to construct diverse classes of nitrogen heterocyclic compounds possessing significant pharmacological activities (Black et al., 2003; Zarghi et al., 2007; Hashem et al., 2007; Husain et al., 2005).



2(*3H*)-furanone 2(*5H*)-furanone 3(*2H*)-furanone

Figure 1 Various isomeric forms of furanone ring.

Quinoline ring system is present in number of bioactive natural products and quite a few are used therapeutically. Quinolines and their synthetic derivatives are reported to exhibit anti-inflammatory and analgesic activities (Husain et al., 2013) in addition to other useful pharmacological activities (Jashim Uddin et al., 2004; Pohle et al., 2001).

Biphenyl based furanones and pyrrolones show interesting antimicrobial and anti-inflammatory activities (Khan and Husain, 2002). Naproxen and Nabumetone are examples of naphthalene containing NSAIDs which are usually indicated in the management of pain and inflammatory conditions. Also it has been reported that several other naphthalene derivatives inhibit cyclooxygenase enzyme, block the synthesis of inflammatory mediators and therefore, display good anti-inflammatory activities (Harrak et al., 2007).

Prompted by these findings, and as a part of our current research interest on furanone derivatives, we thought to prepare compounds having three biological moieties in one i.e. biphenyl and naphthalene based furanones or pyrrolones having quinoline moiety in search of potent lead/drug molecules for anti-inflammatory or antimicrobial therapy. A total of twenty-four title compounds viz. eight furan-2(3*H*)-ones, eight pyrrol-2(3*H*)-ones and eight *N*-benzyl-pyrrol-2(3*H*)-ones were prepared and screened for antibacterial, antifungal, *in vivo* analgesic and anti-inflammatory activities.

2. Experimental

2.1. General

The reagents and solvents used in all experiments were obtained from Merck (Mumbai, India), S.D. Fine (Mumbai, India), CDH (New Delhi) and Qualigens (India). Melting points were recorded in open end capillary tubes using MR-VIS Visual melting point apparatus (LAB India) and are uncorrected. The IR spectra were recorded on Hitachi 150–200 spectrophotometer using KBr. ^{1}H NMR spectra were recorded on Bruker spectrospin DPX-300 MHz in $CDCl_3$ or DMSO using tetramethylsilane (TMS) as an internal reference. Chemical shift (δ) values are reported in parts per million (ppm) while splitting patterns of peaks as singlets, doublet or triplet in proton NMR spectra are indicated by abbreviations *s*, *d*, *t* and *m*, respectively. Mass spectrometry for title compounds was performed on a JEOL JMS-D 300 instrument. Perkin-Elmer 240 analyzer was used to perform elemental analyses (C, H, N) and was found in the range of ±0.4% for each analyzed element. Progress of reaction was monitored on thin-layer chromatography using silica gel G as stationary phase in the solvent system-Toluene:Ethyl acetate:Formic acid (5:4:1, v/v/v) or Petroleum ether:Toluene:Ethyl acetate (5:4:1,

v/v/v) or Ethyl acetate:Hexane (3:7, v/v), and TLC spots were visualized under the UV light.

2.2. Chemical synthesis

2.2.1. Synthesis of 4-substituted phenyl oximes (*1a–d*)

4-Substituted-1-phenylethanone oximes (**1a–d**) were synthesized by reacting different substituted acetophenones (0.1 mol) with hydroxylamine hydrochloride (0.12 mol) in the presence of sodium acetate (0.12 mol) as per the literature method (Alam et al., 2009).

2.2.2. Synthesis of substituted-2-chloroquinoline-3-carbaldehydes (*2a–d*) (Alam et al., 2009)

A freshly distilled phosphorus oxychloride (0.35 mol) was added dropwise with stirring to a previously cooled solution of dimethylformamide (0.15 mol) and then oxime (**1a–d**) (0.05 mol) was added in small portions. The resulting mixture was heated at 60 °C for 16 h, followed by decomposition by pouring into ice cold water (300 mL). The mixture was continuously agitated for 30 min to yield a solid product. The compound so obtained was filtered, dried and recrystallized from ethyl acetate to get TLC pure compound (*2a–d*).

2.2.3. Synthesis of 3-(substituted aroyl) propionic acid (*3,4*)

3-(2-Naphthoyl) propionic acid (**3**) and 3-(biphenyl-4-yl) propionic acid **(4)** were synthesized according to the literature method (Alam et al., 2009).

2.2.4. Synthesis of furan-2(3H)-ones (*5a–h*)

2.2.4.1. General procedure for the synthesis of 3-{(2-chloro-6-substituted quinolin-3-yl) methylidene}-5-(aryl)-furan-2(3H)-one (5a–h). (Alam et al., 2009). To equimolar quantities (0.005 mol) of 3-substituted aroylpropionic acid (**3/4**) and sub stituted-2-chloroquinoline-3-carbaldehydes **(2a–d)**, acetic anhydride was added to just wet the mixture (5–8 drops) and after that 2–3 drops of triethylamine were added. The reaction mixture was refluxed briefly for 10–15 min and then poured over crushed ice with gentle stirring to obtain a colored solid mass. The solid substance after filtration was washed with water, dried and recrystallized from methanol to furnish the desired compounds (**5a–h**). The physical and spectral data of the prepared compounds are summarized in Table 1.

2.2.5. Synthesis of pyrrol-2(3H)-ones (*6a–h*)

2.2.5.1. General procedure for the synthesis of 3-{(2-chloro-6-substituted quinolin-3-yl)methylidene}-5-(aryl)-pyrrol-2(3H)-ones (6a–h). Pyrrolones were prepared by passing the ammonia gas (dry) through anhydrous solution of furanones **5a–h** (1 g) in ethanol. Ethanol was removed under vacuum at the completion of reaction to obtain the solid product. The compounds were crystallized in methanol to get the pure colored crystals of pyrrolones **6a–h**. The physical and spectral data of **6a–h** are presented in Table 2.

2.2.6. Synthesis of N-benzyl-pyrrol-2(3H)-ones (*7a–h*)

2.2.6.1. General procedure for the synthesis of 1-benzyl-3-{(2-chloro-6-substituted quinolin-3-yl)methylidene}-5-(aryl)-pyyrol-2(3H)-ones (7a–h). A mixture of furanone **5a–h** (1.5 mmol) and benzylamine (2 mmol) in dry benzene was refluxed for 1 h. After the completion of reaction, excess benzene was removed under vacuum. The solid so obtained was washed with petroleum ether, dried in air and then refluxed for 1 h in 6 N hydrochloric acid (15 mL). A solid mass was precipitated out on cooling the mixture content which on usual workup and crystallization in methanol yielded the desired *N*-benzyl-pyrrol-2(3*H*)-ones **7a–h**. The physical and spectral data are shown in Table 3.

2.3. Antimicrobial studies

2.3.1. Antibacterial activity

The antibacterial activity of the title compounds was tested against following four strains: *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741), by standard method (Cruickshank et al., 1975). Ciprofloxacin was used as standard antibiotic for comparison of the activity. The compounds showing activity at 100 μg/mL concentration were further tested for their MIC.

2.3.2. Antifungal activity

The antifungal activity of the prepared compounds was evaluated against *Aspergillus niger* and *Aspergillus flavus* fungal strains by the standard poison food technique method (Cruickshank et al., 1975). Fluconazole was used as the standard drug for comparison purpose.

2.4. Pharmacological studies

2.4.1. Animals

Anti-inflammatory and analgesic activities of furanones and their nitrogen analogues were carried out on Wistar rats and Swiss albino mice, respectively, after getting necessary permission of animal's usage from Kurukshetra University animal ethics committee (Regd. No. 563/02/a/CPCSEA). Albino rats 160–200 g and male mice weighing 25–30 g were housed in polypropylene cages in group of six and acclimatized to the conditions for 48 h before the commencement of study.

2.4.2. Anti-inflammatory activity

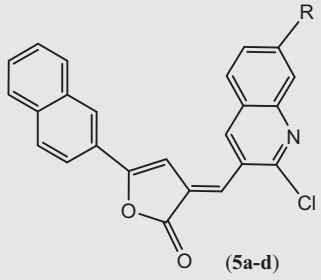
Anti-inflammatory activity of the title compounds was evaluated by the method of Winter et al. (1962) in Wistar rats of either sex, weighing 160–200 g. The percentage inhibition of edema was calculated at 1 h, 2 h, 3 h and 4 h and the results were compared with the standard drug, diclofenac.

2.4.3. Analgesic activity

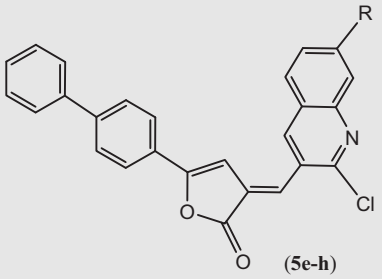
Eight compounds which displayed good anti-inflammatory activity (> 53% inhibition) were selected for evaluation of peripheral and central analgesic activity in mice by two methods viz. acetic acid induced writhing method and tail immersion method (Seigmund et al., 1957).

2.4.3.1. Acetic acid induced constrictions method. The peripheral analgesic activity of selected compounds was evaluated by acetic acid induced writhing method. Aqueous acetic acid was used to induce writhing in Swiss Albino mice (20–30 g)

Table 1 Physical data and spectral data of 3-[(2-chloro-6-substituted-quinolin-3-yl)methylene]-5-(aryl)furan-2(3*H*)-one (**5a–h**).



(5a-d)



(5e-h)

Compd	—R	Physical data and spectral data
5a	—H	3-[(2-Chloroquinolin-3-yl) methylene]-5-(naphthalene-2-yl)furan-2(3*H*)-one: Yield 65%; m.p. 221–222 °C, R_f 0.92, IR (KBr) cm^{-1} 1763 (C═O), 1566 (ArC═C), 1063 (ArC—N), 847 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.92 (s, 1H, βH), 7.25 (s, 1H, olefinic H), 7.63–8.25 (complex m, 12H, aryl protons), MS (*m*/*z*): 383 (M^+), 384 (M + 1), 385 (M + 2); Anal. Calcd. for $C_{24}H_{14}ClNO_2$: C, 75.10; H, 3.68; N, 3.65; Found: C, 75.22; H, 3.44; N, 3.52
5b	—Cl	3-[(2,6-Dichloroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)furan-2(3*H*)-one: Yield 57%; m.p. 245–246 °C, R_f 0.88, IR (KBr) cm^{-1} 1767 (C═O), 1558 (ArC═C), 1066 (ArC—N), 849 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.60 (s, 1H, βH), 7.29 (s, 1H, olefinic H), 7.42–8.20 (complex m, 11H, aryl protons), MS (*m*/*z*): 417 (M^+), 418 (M + 1), 419 (M + 2); Anal. Calcd. for $C_{24}H_{13}Cl_2NO_2$: C, 68.92; H, 3.13; N, 3.35; Found: C, 68.75; H, 3.05; N, 3.23
5c	—CH_3	3-[(2-Chloro-6-methylquinolin-3-yl)methylene]-5-(naphthalene-2-yl)furan-2(3*H*)-one: Yield 61%; m.p 214–215 °C, R_f 0.91, IR (KBr) cm^{-1} 1759 (C═O), 1561 (ArC═C), 1061 (ArC—N), 852 (ArC—H). ^{1}H NMR ($CDCl_3$): 2.18 (s, 3H, CH_3), 6.78 (s, 1H, βH), 7.22 (s, 1H, olefinic H), 7.28–8.26 (complex m, 11H, aryl protons), MS (*m*/*z*): 397 (M^+), 398 (M + 1), 399 (M + 2), Anal. Calcd. for $C_{25}H_{16}ClNO_2$: C, 75.47; H, 4.05; N, 3.52, Found: C, 75.63; H, 3.83; N, 3.44
5d	—NO_2	3-[(2-Chloro-6-nitroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)furan-2(3*H*)-one: Yield 59%; m.p 257–258 °C, R_f 0.85, IR (KBr) cm^{-1} 1774 (C═O), 1570 (ArC═C), 1063 (ArC—N), 857 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.81 (s, 1H, βH), 7.28 (s, 1H, olefinic H), 7.45–8.18 (complex m, 11H, aryl protons); MS (*m*/*z*): 428 (M^+), 429 (M + 1), 430 (M + 2) Anal. Calcd. for $C_{24}H_{13}ClN_2O_4$: C, 67.22; H, 3.06; N, 6.53; Found: C, 67.05. H, 3.28; N, 6.25
5e	—H	3-[(2-Chloroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)furan-2(3*H*)-one: Yield 63%; m.p 120–122 °C, R_f 0.86, IR (KBr) cm^{-1} 1769 (C═O), 1563 (ArC═C), 1059 (ArC—N), 865 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.88 (s, 1H, βH), 7.37 (s, 1H, olefinic proton), 7.53–8.23 (m, 14H, aryl protons), MS (*m*/*z*): 409 (M^+), 410 (M + 1), 411 (M + 2). Anal. Calcd. for $C_{26}H_{16}ClNO_2$: C, 76.19; H, 3.93; N, 3.42; Found: C, 76.35; H, 4.03; N, 3.37
5f	—Cl	3-[(2,6-Dichloroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)furan-2(3*H*)-one: Yield 46%; m.p 246 °C, R_f 0.76, IR (KBr) cm^{-1} 1713 (C═O), 1518 (ArC═C), 1038 (ArC—N), 824 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.85 (s, 1H, βH), 7.40 (s, 1H, olefinic proton), 7.46–8.35 (m, 13H, aryl protons), MS (*m*/*z*): 444 (M^+), 445 (M + 1), 446 (M + 2); Anal. Calcd. for $C_{26}H_{15}Cl_2NO_2$: C, 70.28; H, 3.40; N, 3.15; Found: C, 70.13; H, 3.32; N, 3.09
5g	—CH_3	3-[(2-Chloro-6-methylquinolin-3-yl)methylene]-5-(biphenyl-4-yl)furan-2(3*H*)-one: Yield 58%; m.p 154 °C, R_f-0.74, IR (KBr) cm^{-1} 1752 (C═O), 1556 (ArC═C), 1054 (ArC—N), 829 (ArC—H). ^{1}H NMR ($CDCl_3$): 2.13 (s, 3H, CH_3), 6.89 (s, 1H, βH), 7.33 (s, 1H, olefinic proton), 7.38–8.24 (m, 13H, aryl protons), MS (*m*/*z*): 423 (M^+), 424 (M + 1), 425 (M + 2); Anal. Calcd. for $C_{27}H_{18}ClNO_2$: C, 76.50; H, 4.28; N, 3.30; Found: C, 76.26; H, 4.18; N, 3.43.
5h	—NO_2	3-[(2-Chloro-6-nitroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)furan-2(3*H*)-one: Yield 65%; m.p. 184–186 °C, R_f-0.71, IR (KBr) cm^{-1} 1767 (C═O), 1553 (ArC═C), 1051 (ArC—N), 838 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.91 (s, 1H, βH), 7.39 (s, 1H, olefinic proton), 7.46–8.31 (m, 13H, aryl protons), MS (*m*/*z*): 454 (M^+), 455 (M + 1), 456 (M + 2); Anal. Calcd. for $C_{26}H_{15}ClN_2O_4$: C, 68.65; H, 3.32; N, 6.16; Found: C, 68.43; H, 3.45; N, 6.07

of either sex which were divided into groups of six animals in each. The analgesic activity was calculated by using the following formula:

$$\%\text{Protection} = \{(Wc - Wt)/Wc\} \times 100$$

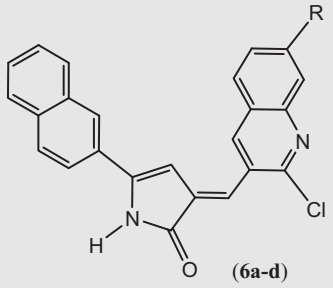
where Wc = mean number of writhing of control group, and Wt = mean number of writhing of test group.

2.4.3.2. Tail immersion method. The Mice used in the writhing test were again used for tail immersion method after washing period (Seigmund et al., 1957). Mice of each group were placed in a suitable restrainer such that their tail protrudes outside. The protruding tail (up to 5 cm) is dipped into hot water (55 °C) and the time taken by the mice to withdraw its tail out of water is recorded as the reaction time. Readings were taken at 1 h and 2 h after the dosing.

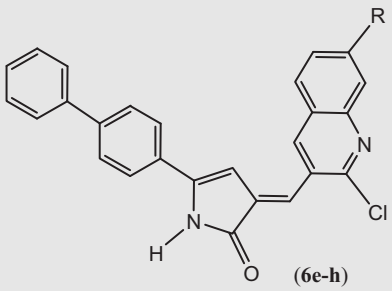
2.4.4. Acute ulcerogenic activity

Acute ulcerogenic studies were performed in Albino rats as per the method of Cioli et al. (1979). The mucosal damage was examined at the end of study and ulcerogenic potential of the compounds was calculated by comparing the average score of treatment group with the mean score of rats in control group.

Table 2 Physical data and spectral data of 3-[(2-chloro-6-substituted-quinolin-3-yl)methylene]-5-(aryl)-1*H*-pyrrol-2(3*H*)-one (**6a–f**).



(**6a-d**)



(**6e-h**)

Compd	—R	Physical and spectral data
6a	—H	3-[(2-Chloroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 78%, m.p. 280 °C, R_f-0.79, IR (KBr, cm^{-1}); 3410 (NH), 1696 (C═O), 1582 (ArC═C), 1033 (ArC—N), 802 (ArC—H). ^{1}H NMR ($CDCl_3$) δ = 6.46 (s, 1H, βH), 7.08 (s, 1H, olefinic H), 7.41–8.13 (complex m, 13H, 12 aryl protons + NH). MS[EI] *m*/*z* 382 (M^+), 383 (M + 1), 384 (M + 2). Elemental Analysis.Calcd. $C_{24}H_{15}ClN_2O$: C, 75.29; H, 3.95; N, 7.32; found: C, 75.42; H, 3.87; N, 7.25
6b	—Cl	3-[(2,6-Dichloroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 74%; m.p 205 °C, R_f-0.80, IR (KBr) cm^{-1}–3443 (NH), 1706 (C═O), 1602 (ArC═C), 1057 (ArC—N), 808 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.32 (s, 1H, βH), 7.11 (s, 1H, olefinic H), 7.38–8.15 (complex m, 13H, 12 aryl protons + NH); MS (*m*/*z*): 416 (M^+), 417 (M + 1), 418 (M + 2); Anal. Calcd. for $C_{24}H_{14}Cl_2N_2O$: C, 69.08; H, 3.38; N, 6.71; Found: C, 68.86; H, 3.34; N, 6.84
6c	—CH_3	3-[(2-Chloro-6-methylquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 71%; m.p. 240 °C, R_f-0.78, IR (KBr) cm^{-1} 3388 (NH), 1683 (C═O), 1607 (ArC═C), 1019 (ArC—N), 801 (ArC—H). ^{1}H NMR ($CDCl_3$): 2.23 (s, 3H, CH_3), 6.54 (s, 1H, βH), 7.12 (s, 1H, olefinic H), 7.31–8.18 (complex m, 12H, 11 aryl protons + NH); MS (*m*/*z*): 396 (M^+), 397 (M + 1), 398 (M + 2). Anal. Calcd. for $C_{25}H_{17}ClN_2O$: C, 75.66; H, 4.32; N, 7.06; Found: C, 75.46; H, 4.27; N, 7.22
6d	—NO_2	3-[(2-Chloro-6-nitroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 68%; m.p. 281 °C, R_f-0.73, IR (KBr) cm^{-1} 3435 (NH), 1692 (C═O), 1591 (ArC═C), 1036 (ArC—N), 814 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.63 (s, 1H, βH), 7.22 (s, 1H, olefinic H), 7.25–8.29 (complex m, 12H, 11 aryl protons + NH); MS (*m*/*z*): 427 (M^+), 428 (M + 1), 429 (M + 2), Anal. Calcd. for $C_{24}H_{14}ClN_3O_3$: C, 67.38; H, 3.30; N, 9.82; Found: C, 67.45; H, 3.21; N, 9.65
6e	—H	3-[(2-Chloroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 66%; m.p 148–150° C, R_f-0.76, IR (KBr) cm^{-1} 3392 (NH), 1687 (C═O), 1590 (ArC═C), 1018 (ArC—N), 792 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.28 (s, 1H, βH), 7.13 (s, 1H, olefinic H), 7.15–8.29 (complex m, 15H, 14 aryl protons + NH); MS (*m*/*z*): 408 (M^+), 409 (M + 1), 410 (M + 2); Anal. Calcd. for $C_{26}H_{17}ClN_2O$: C, 76.37; H, 4.19; N, 6.85, Found: C, 76.47; H, 4.14; N, 6.61
6f	—Cl	3-[(2,6-Dichloroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 70%; R_f-0.67, IR (KBr) cm^{-1} 3451 (NH), 1689 (C═O), 1601 (ArC═C), 1026 (ArC—N), 813 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.36 (s, 1H, βH), 7.12 (s, 1H, olefinic H), 7.24–8.31 (complex m, 14H, 13 aryl protons + NH); MS (*m*/*z*): 442 (M^+), 443 (M + 1), 444 (M + 2); Anal. Calcd. for $C_{26}H_{16}Cl_2N_2O$: C, 70.44; H, 3.64; N, 6.32; Found: C, 70.31; H, 3.58; N, 6.25
6g	—CH_3	3-[(2-Chloro-6-methylquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 72%; R_f-0.71, IR (KBr) cm^{-1} 3441 (NH), 1696 (C═O), 1594 (ArC═C), 1019 (ArC—N), 788 (ArC—H). ^{1}H NMR ($CDCl_3$): 2.25 (s, 3H, CH_3), 6.31 (s, 1H, βH), 7.12 (s, 1H, olefinic H), 7.17–8.20 (complex m, 14H, 13 aryl protons + NH); MS (*m*/*z*): 422 (M^+), 423 (M + 1), 424 (M + 2); Anal. Calcd. for $C_{27}H_{19}ClN_2O$: C, 76.68; H, 4.53; N, 6.62; Found: C, 76.54; H, 4.49; N, 6.54
6h	—NO_2	3-[(2-Chloro-6-nitroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 65%; R_f-0.70, IR (KBr) cm^{-1} 3435 (NH), 1708 (C═O), 1611 (ArC═C), 1037 (ArC—N), 805 (ArC—H). ^{1}H NMR ($CDCl_3$): 6.36 (s, 1H, βH), 7.08 (s, 1H, olefinic H), 7.12–8.34 (complex m, 14H, 13 aryl protons + NH); MS (*m*/*z*): 453 (M^+), 454 (M + 1), 455 (M + 2); Anal. Calcd. for $C_{26}H_{16}ClN_3O_3$: C, 68.80; H, 3.55; N, 9.26; Found: C, 68.63; H, 3.28; N, 9.55

2.5. Docking studies

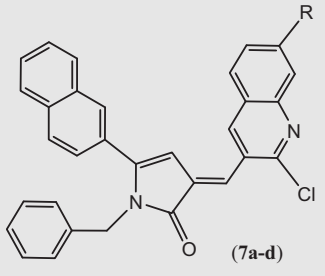
The ligand dataset was virtually screened with the protein targets using Molegro software (MVD 4.2) and the binding energy values were analyzed for each docked conformation (William et al., 2008; Reyes and Kollman, 2000). Conformations having low energy and exhibited favorable hydrogen bonding with the amino acids side chain and its amide nitrogen were considered (Table 7). Binding energies of the protein–ligand interactions are of significant importance because they tell us how well the ligand binds to the target macromolecule. Docking simulations of furanones against 3LN1 protein target lead to the identification of few potential compounds which were evaluated based on the binding compatibility [docked energy (kcal/mol)] with the receptor.

3. Results and discussion

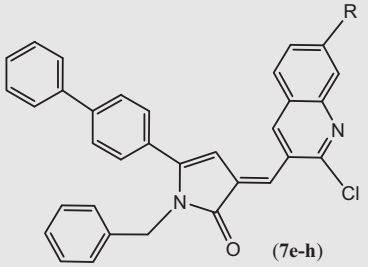
3.1. Chemistry

The title compounds were prepared as per the protocol outlined in Scheme 1. The present work involved clubbing of furanone and pyrrolones with quinoline moiety to obtain newer effective compounds. The synthetic methodology involves the synthesis of quinoline-3-carbaldehyde derivatives (***2a–d***) by reacting *N,N*-dimethylformamide (DMF) with acetophenone

Table 3 Physical and spectral data of 1-benzyl-3-{(2-chloro-6-substituted quinolin-3-yl)methylidene}-5-(aryl)-1*H*-pyyrol-1(3*H*)-one (**7a–h**).



(**7a-d**)

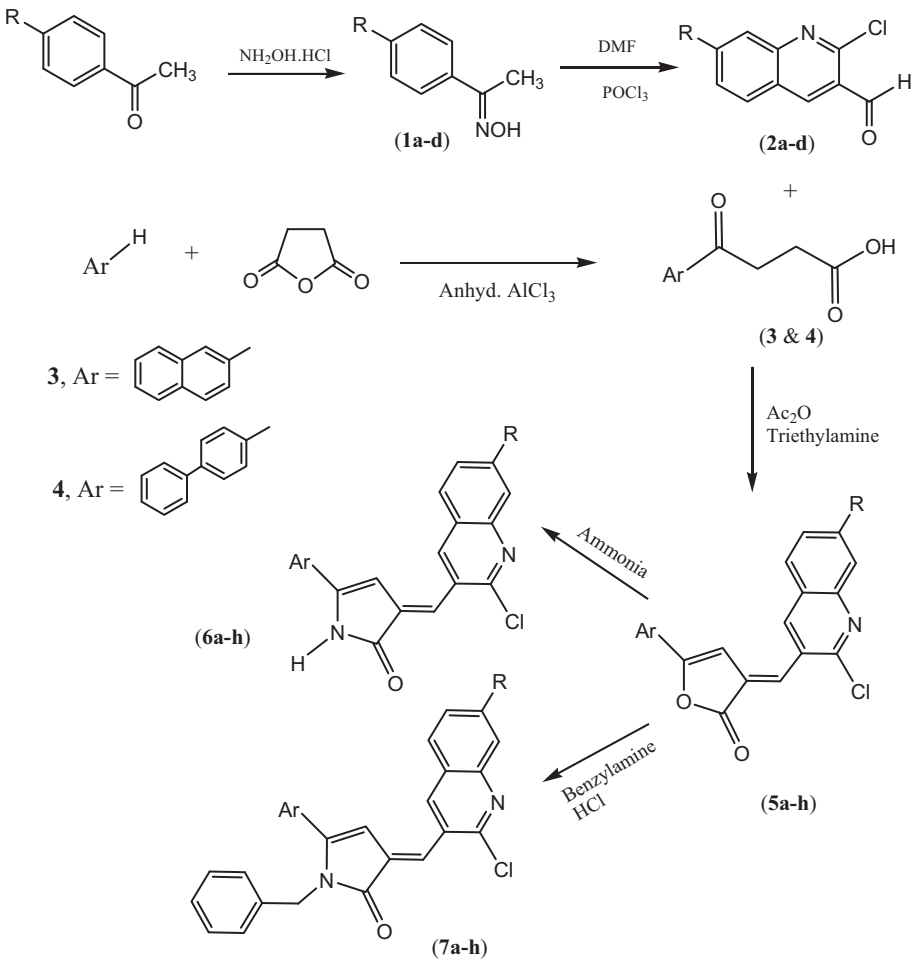


(**7e-h**)

Compd	—R	Physical and spectral data
7a	—H	1-Benzyl-3-[(2-chloroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 74%; m.p. 183 °C, R_f-0.87, IR (KBr) cm^{-1} 1753 (C═O), 1603 (ArC═C), 1038 (ArC—N), 808 (ArC—H). ^1H NMR ($CDCl_3$): 4.89 (s, 2H, CH_2), 6.48 (s, 1H, βH), 7.09–8.18 (complex m, 18H, 17 aryl protons + olefinic H), MS (*m*/*z*): 472 (M^+), 473 (M + 1), 474 (M + 2); Anal. Calcd. for $C_{31}H_{21}ClN_2O$: C, 78.72; H, 4.48; N, 5.92; Found: C, 78.52; H, 4.53; N, 5.78
7b	—Cl	1-Benzyl-3-[(2,6-dichloroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 70%; m.p. 168–170 °C, R_f-0.82, IR (KBr) cm^{-1} 1748 (C═O), 1595 (ArC═C), 1033 (ArC—N), 812 (ArC—H) ^1H NMR ($CDCl_3$): 4.89 (s, 2H, CH_2), 6.54 (s, 1H, βH), 7.09–8.18 (complex m, 18H, 17 aryl protons + olefinic H), MS (*m*/*z*): 506 (M^+), 507 (M + 1), 508 (M + 2); Anal. Calcd. for $C_{31}H_{20}Cl_2N_2O$: C, 73.38; H, 3.97; N, 5.52; Found: C, 73.13; H, 3.83; N, 5.34
7c	—CH_3	1-Benzyl-3-[(2-chloro-6-methylquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 72%; m.p. 176 °C, R_f-0.87, IR (KBr) cm^{-1} 1742 (C═O), 1606 (ArC═C), 1041 (ArC—N), 815 (ArC—H). ^1H NMR ($CDCl_3$): 2.38 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 6.46 (s, 1H, βH), 7.18–8.25 (complex m, 17H, 16 aryl protons + olefinic H), MS (*m*/*z*): 486 (M^+), 487 (M + 1), 488 (M + 2). Anal. Calcd. for $C_{32}H_{23}ClN_2O$: C, 78.92; H, 4.76; N, 5.75; Found: C, 78.57; H, 4.65; N, 5.67
7d	—NO_2	1-Benzyl-3-[(2-chloro-6-nitroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 66%; m.p. 164–166 °C, R_f-0.78, IR (KBr) cm^{-1} 1739 (C═O), 1611 (ArC═C), 1046 (ArC—N), 821 (ArC—H). ^1H NMR ($CDCl_3$): 4.88 (s, 2H, CH_2), 6.44 (s, 1H, βH), 7.09–8.18 (complex m, 18H, 17 aryl protons + olefinic H), MS (*m*/*z*): 517 (M^+), 518 (M + 1), 519 (M + 2); Anal. Calcd. for $C_{31}H_{20}ClN_3O_3$: C, 71.88; H, 3.89; N, 8.11; Found: C, 71.78; H, 3.65; N, 8.25
7e	—H	1-Benzyl-3-[(2-chloroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 63%; m.p 154–155 °C, R_f-0.79, IR (KBr) cm^{-1} 1746 (C═O), 1592 (ArC═C), 1037 (ArC—N), 807 (ArC—H). ^1H NMR ($CDCl_3$): 4.89 (s, 2H, CH_2), 6.26 (s, 1H, βH), 7.08–8.11 (complex m, 20H, 19 aryl protons + olefinic H), MS (*m*/*z*): 498 (M^+), 499 (M + 1), 500 (M + 2), Anal. Calcd. for $C_{33}H_{23}ClN_2O$: C, 79.43; H, 4.65; N, 5.61; Found: C, 79.52; H, 4.46; N, 5.52
7f	—Cl	1-Benzyl-3-[(2,6-dichloroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 71%; m.p 142–144 °C, R_f-0.81, IR (KBr) cm^{-1}-1742 (C═O), 1598 (ArC═C), 1039 (ArC—N), 811 (ArC—H). ^1H NMR ($CDCl_3$): 4.89 (s, 2H, CH_2), 6.23 (s, 1H, βH), 7.19–8.16 (complex m, 20H, 19 aryl protons + olefinic H), MS (*m*/*z*): 532 (M^+), 533 (M + 1), 534 (M + 2), Anal. Calcd. for $C_{33}H_{22}Cl_2N_2O$: C, 74.30; H, 4.16; N, 5.25; Found: C, 74.47; H, 4.25; N, 5.17
7g	—CH_3	1-Benzyl-3-[(2-chloro-6-methylquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 71%; m.p 170 °C, R_f-0.72, IR (KBr) cm^{-1} 1737 (C═O), 1599 (ArC═C), 1034 (ArC—N), 796 (ArC—H). ^1H NMR ($CDCl_3$): 2.36 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 6.26 (s, 1H, βH), 7.03–8.12 (complex m, 20H, 19 aryl protons + olefinic H), MS (*m*/*z*): 512 (M^+), 513 (M + 1), 514 (M + 2), Anal. Calcd. for $C_{34}H_{25}ClN_2O$: C, 79.60; H, 4.91; N, 5.46; Found: C, 79.41; H, 5.14; N, 5.18
7h	—NO_2	1-Benzyl-3-[(2-chloro-6-nitroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1*H*-pyrrol-2(3*H*)-one: Yield 71%; m.p. 142–144 °C, R_f-0.81, IR (KBr) cm^{-1} 1744 (C═O), 1613 (ArC═C), 1046 (ArC—N), 818 (ArC—H). ^1H NMR ($CDCl_3$): 4.89 (s, 2H, CH_2), 6.23 (s, 1H, βH), 7.19–8.16 (complex m, 20H, 19 aryl protons + olefinic H), MS (*m*/*z*): 543 (M^+), 544 (M + 1), 545 (M + 2), Anal. Calcd. for $C_{33}H_{22}ClN_3O_3$: C, 72.86; H, 4.08; N, 7.72; Found: C, 72.63; H, 3.84; N, 7.57

oxime (**1a–d**) in the presence of phosphorus oxychloride ($POCl_3$). The reaction proceeds via Beckmann rearrangement followed by Vilsmeier–Haack formylation. The 3-aroyl propionic acids (**3**,**4**) were prepared by Friedel–Craft acylation of naphthalene and biphenyl with succinic anhydride. Condensation of quinolone-3-carbaldehyde (**2a–d)** with 3-aroyl propionic acid (**3,4**) in the presence of acetic anhydride resulted in the formation of 3-{(2-chloro-6-substituted quinolin-3-yl) methylidene}-5-(aryl)-furan-2(3*H*)-one (**5a–h**). The 3-{(2-chloro-6-substituted quinolin-3-yl) methylidene}-5-(aryl)-pyrrol-2(3*H*)-ones (**6a–h**) were prepared by reacting furan-2(*3H*)-ones (**5a–h**) with dry ammonia in absolute ethanol. The 1-benzyl-3-{(2-chloro-6-substituted quinolin-3-yl) methylidene}-5-(aryl)-pyyrol-2(3*H*)-ones (**7a–h**) were obtained by reacting furan-2(3*H*)-ones with benzylamine in dry benzene to give γ-ketobenzylamides, which were then lactamized in 6 N HCl to furnish the corresponding *N*-benzyl-2(3*H*)-pyrrolones (**7a–h**). The chemical structures of the title compounds were characterized by IR, ^1H NMR, Mass spectral data and are well supported by elemental analysis.

The infrared spectral studies (IR; cm^{-1}) of furan-2(3*H*)-ones **5a–h** showed bands at 1774–1713 (lactone C═O); 1570–1518 (ArC═C), 1066–1038 (ArC—N), and 865–824 (ArC—H). IR band for Pyrrol-2(3*H*)-one **6a–h** appeared at 3451–3388 (pyrrolone N—H), 1708–1683 (C═O); 1611–1582 (ArC═C) and 814–788 (ArC—H). IR spectra of *N*-



Scheme 1 Protocol for synthesis of title compounds.

Benzylpyrrol-2(3*H*)-ones **7a–h** gave bands at 1753–1737 (lactone C═O); 1613–1592 (ArC═C), 1046–1033 (ArC─N), and 821–796 (ArC─H). In ^{1}H NMR spectra, signal at around δ 6.6 indicates the formation of furan ring. The absence of aldehydic proton and presence of alkenic proton further indicate the conversion of aldehydic group to the desired compound. The δ values were calculated using incremental parameters for the hydrogen (semicyclic double bond) which indicated an (*E*)-configuration. The Mass spectra of the title compounds displayed M^+ peak in reasonable intensities. The molecular ion peak and isotopic peaks and fragment peaks were quite clear due to the presence of chlorine atom(s) in all the title compounds. The physical and spectral data of all the synthesized compounds (**5a–h**, **6a–h** and **7a–h**) are presented in Tables 1–3.

3.2. Antimicrobial activity

All the three series of synthesized compounds (**5a–h**, **6a–h** and **7a–h**) were screened for the antimicrobial activity against few selected bacterial and fungal strains.

3.2.1. Antibacterial and antifungal activity

All the screened compounds showed variable antimicrobial activity against the tested microbes. The results of antibacterial testing indicate that four pyrrolone compounds **6b**, **6d**, **6f** and **6h** are highly active against *S. aureus* and three compounds **6b, 6d** and **6f** against *B. subtilis,* with MIC 6.25 μg/mL. The most potent antibacterial compounds among furanones and N-benzyl pyrrolones against *S. aureus* were found to be **5b**, **5d**, **5f** and **7f**, respectively with MIC of 12.5 μg/mL. However, compounds **5a**, **5c**, **5g**, **7e** and **7g** did not show any inhibition against the gram negative bacteria *E. coli* and *P. aeruginosa.* The most potent compounds against *E. coli* and *P. aeruginosa* were observed to be **5d**, **6d**, **7b** and **6d**, respectively with a MIC of 12.5 μg/mL. Compounds **6d** and **6f** also exhibited significant antifungal activity against *A. niger* with MIC 6.25 μg/mL. Their activity was at par with the standard drugs, ciprofloxacin or fluconazole which also had MIC of 6.25 μg/mL. In general, majority of the tested compounds were found to be less active against the *E. coli, P. aeruginosa* and *A. flavus*. Results of antibacterial and antifungal activity are summarized in Table 4.

A closer look at the results revealed that the title compounds possess better antifungal activity as compared to antibacterial activity. Among all the prepared compounds, **6d** and **6f** were found to be the most promising antibacterial and antifungal agents. It was interesting to note that substitution of oxygen atom with the nitrogen i.e. converting furans into corresponding pyrrolones, significantly enhances the antimicrobial activity; however, introduction of benzylamine

Table 4 Antibacterial and antifungal activity (MIC, μg/mL) of synthesized compounds.

Compd	Antibacterial activity				Antifungal activity	
	S. aureus	*E. coli*	*P. aeruginosa*	*B. subtilis*	*A. niger*	*A. flavus*
5a	50	–	–	50	50	–
5b	12.5	25	50	12.5	25	50
5c	25	–	–	50	50	> 100
5d	12.5	12.5	25	25	25	25
5e	25	–	–	50	25	50
5f	12.5	50	> 100	25	25	25
5g	25	–	–	50	50	50
5h	25	50	–	> 100	25	25
6a	25	–	50	25	25	25
6b	6.25	50	50	6.25	12.5	12.5
6c	12.5	–	50	12.5	25	12.5
6d	6.25	12.5	12.5	6.25	6.25	12.5
6e	12.5	–	50	50	50	25
6f	6.25	50	25	6.25	6.25	12.5
6g	25	50	50	12.5	25	12.5
6h	6.25	25	25	12.5	25	25
7a	50	25	–	> 100	50	> 100
7b	25	12.5	> 100	25	50	25
7c	25	50	–	50	50	25
7d	25	25	> 100	25	25	50
7e	50	–	–	–	50	> 100
7f	12.5	25	50	12.5	25	50
7g	25	–	–	50	25	50
7h	25	50	50	–	50	> 100
Standard-1[a]	6.25	6.25	6.25	nt	nt	Nt
Standard-2[a]	Nt	Nt	Nt	6.25	6.25	6.25

– Indicates microbes are resistant to the compounds > 100 μg/mL; nt = not tested.
[a] Standard-1 = Ciprofloxacin, Standard-2 = Fluconazole; MIC = minimum inhibitory concentration.

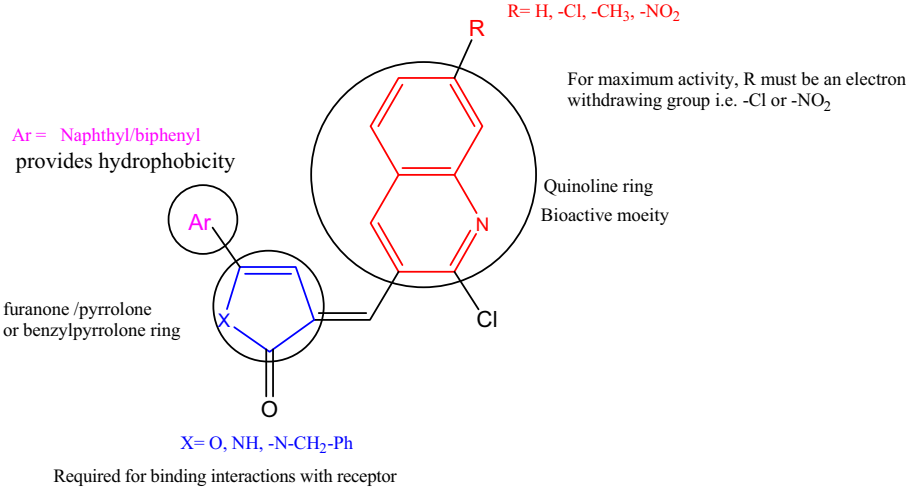


Figure 2 General structure of synthesized compounds.

moiety in place of oxygen atom (benzylpyrrolones) in the furanone ring leads to decreased antimicrobial activity. This change in activity may be due to proton donor capacity of pyrrolones.

Thus, based on the above results, the following structure activity relationship (SAR) can be proposed for the synthesized compounds (Fig. 2):

1. Presence of a chloro group on the quinoline nucleus with attached biphenyl ring was found to increase activity of pyrrolone toward inhibition of *S. aureus* and *B. subtilis*.
2. Presence of N-benzyl pyrrolone with attached biphenyl ring and quinoline ring was found to be highly active. But any substitution in quinoline ring at 6-position causes decrease in activity.

3. Presence of a chloro group on the quinoline nucleus with attached naphthalene ring was found to increase activity of pyrrolone toward inhibition of *A. niger and A. flavus*.

3.3. Anti-inflammatory activity

The results of anti-inflammatory activity presented in Table 5 showed that furanones (**5a–h**) inhibited carrageenan induced edema from 56% to 63%, pyrrolones (**6a–h**) 36–53%, *N*-benzyl pyrrolones (**7a–h**) 40–71% in comparison with the standard diclofenac, 92%. Compound 3-[(2-Chloro-6-nitroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)furan-2(3H)-one **(5h)** among furanones (63% inhibition), **6b** among pyrrolones (53% inhibition) and 1-benzyl-3-[(2-chloro-6-nitroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1H-pyrrol-2(*3H*)-one **(7d)** among N- benzyl pyrrolones (71% inhibition) were observed to be the most potent compounds. Thus, it could be concluded that furanones are potent anti-inflammatory agents and substitution of oxygen atom of furanone ring with -NH-(pyrrolone) leads to markedly decreased anti-inflammatory activity, while replacement by benzylamine moiety (*N*-benzyl pyrrolone) enhanced the anti-inflammatory action (Table 5). Thus, it could be concluded that to exhibit the potent anti-inflammatory activity, nitrogen atom of pyrrolone ring should be tertiary. Among 1-benzyl-2(3*H*)-Pyrrolones (**7a–h**), the maximum anti-inflammatory activity was shown by **7d** and closely followed by 1-benzyl-3-[(2,6-dichloroquinolin-3-yl)methylene]-5-(naphthalene-2-yl)-1H-pyrrol-2(*3H*)-one **7b** with 71% and 70% inhibition respectively. The other two compounds, 1-Benzyl-3-[(2,6-dichloroquinolin-3-yl)methylene]-5-(biphenyl-4-yl)-1H-pyrrol-2(*3H*)-one **7h** and 1-Benzyl-3-[(2-chloro-6-nitroquinolin-3-yl) methylene]-5-(biphenyl-4-yl)-1H-pyrrol-2(*3H*)-one **7f** also showed good inhibition of 69% and 64% respectively.

3.4. Analgesic activity

A total of eight test compounds **(5b, 5d, 5h, 6b, 7b, 7d, 7f, 7h)** displaying good inhibition of rat paw edema was selected for further investigation of their analgesic and ulcerogenic actions. Acetic acid induced writhing method and tail-immersion method were used to evaluate peripherally and centrally mediated analgesic effects of the selected compounds. The results of central analgesic activity by tail immersion method indicated a gradual increase in reaction time at 1 h and 2 h, respectively (Table 6). The tested compounds showed reaction time of 1–8–5.5 min at 1 h while it was much higher at 2 h (3.4–6.5 min). The compounds **5d, 5h** and **7h** showed very significant analgesic activity (4.4, 4.3 and 5.5 min) comparable to that of standard at 1 h (8.2 min) while the compounds **5d, 5h, 7f** and **7h** also showed good activity (6.5, 6.2, 6.2 and 6.4 min) at 2 h in tail immersion method. It was interesting to note that **5d**, **5h**, **7f** and **7h** were also found to be the most potent peripheral acting analgesic agents (Table 6), suggesting that these compounds act both peripherally and centrally to abolish pain. The percent protection against the acetic acid induced constrictions for **5d**, **5h**, **7f** and **7h** was in the range

Table 5 Anti-inflammatory activity of the title compounds (**5a–h, 6a–h** and **7a–h**).

Compound	% Inhibition ± SEM[b]			
	After 1 h	After 2 h	After 3 h	After 4 h
Control	–	–	–	–
Standard	84 ± 0.02**	90 ± 0.03**	95 ± 0.02**	92 ± 0.03**
5a	67 ± 0.14**	62 ± 0.11**	46 ± 0.26	57 ± 0.27**
5b	74 ± 0.18**	67 ± 0.06**	39 ± 0.29	59 ± 0.13**
5c	69 ± 0.12**	66 ± 0.25**	47 ± 0.17	59 ± 0.12**
5d	79 ± 0.12**	61 ± 0.15**	56 ± 0.25*	59 ± 0.21**
5e	63 ± 0.09*	60 ± 0.22**	53 ± 0.18	59 ± 0.12**
5f	63 ± 0.13**	58 ± 0.21**	53 ± 0.17	58 ± 0.12**
5g	67 ± 0.12**	62 ± 0.11**	46 ± 0.33	56 ± 0.06**
5h	73 ± 0.08**	58 ± 0.15**	50 ± 0.13	63 ± 0.12**
6a	64 ± 0.06*	53 ± 0.18**	49 ± 0.10	36 ± 0.32
6b	62 ± 0.09*	66 ± 0.18**	53 ± 0.17	53 ± 0.18*
6c	32 ± 0.02	55 ± 0.22**	47 ± 0.24	42 ± 0.20
6d	58 ± 0.25*	60 ± 0.22**	29 ± 0.16	48 ± 0.15*
6e	38 ± 0.21	48 ± 0.21**	44 ± 0.33	35 ± 0.32
6f	48 ± 0.13	63 ± 0.27**	25 ± 0.34	42 ± 0.20
6g	52 ± 0.08*	48 ± 0.24**	25 ± 0.17	36 ± 0.19
6h	58 ± 0.19*	58 ± 0.05**	50 ± 0.18	48 ± 0.15*
7a	52 ± 0.13*	62 ± 0.27**	28 ± 0.34	57 ± 0.13**
7b	84 ± 0.06**	74 ± 0.21**	70 ± 0.23*	70 ± 0.21**
7c	78 ± 0.12**	71 ± 0.28**	57 ± 0.25*	61 ± 0.21**
7d	83 ± 0.06**	73 ± 0.15**	66 ± 0.26	71 ± 0.22**
7e	78 ± 0.12**	51 ± 0.11**	44 ± 0.08	41 ± 0.08
7f	84 ± 0.12	74 ± 0.19**	63 ± 0.14*	64 ± 0.21**
7g	69 ± 0.03**	54 ± 0.14**	30 ± 0.20	40 ± 0.16
7h	36 ± 0.13	65 ± 0.24**	58 ± 0.34*	69 ± 0.15**

Data are arranged as mean ± SEM ANOVA followed by Dunnet's *t* test where $^{*}p < 0.05$; $^{**}p < 0.01$.

Table 6 Analgesic and ulcerogenic activity of compounds **5b, 5d, 5h, 6b, 7b, 7d, 7f** and **7h**.

Compound	Central analgesic activity [tail immersion (reaction time in min)]		Peripheral analgesic activity (writhing test)		Ulcerogenic activity (severity index)
	1 h	2 h	No. of writhing	% Protection	
Control	1.4 ± 0.2	2.8 ± 0.2	42 ± 11.6	0	0.00 ± 0.00
Diclofenac	8.2 ± 0.2**	8.8 ± 0.2**	4.2 ± 1.0**	90	0.86 ± 0.28
5b	3.2 ± 0.2*	4.4 ± 0.5	26.2 ± 4.1	37.61	0.33 ± 0.35*
5d	4.4 ± 0.6**	6.5 ± 0.2 *	6.0 ± 2.2**	85.71	0.30 ± 0.31*
5h	4.3 ± 0.4**	6.2 ± 1.5*	7.6 ± 2.3**	81.9	0.40 ± 0.36
6b	1.8 ± 0.2	4.2 ± 0.6	13.8 ± 4.4*	67.14	0.43 ± 0.33
7b	2.4 ± 0.3	3.4 ± 0.2	25.4 ± 4.8	39.52	0.36 ± 0.35*
7d	3.2 ± 0.4*	5.4 ± 1.9	21.0 ± 10.0*	50	0.40 ± 0.32
7f	3.2 ± 0.2*	6.2 ± 1.5*	12.8 ± 3.8**	69.52	0.53 ± 0.12
7h	5.5 ± 0.3**	6.4 ± 0.3*	10.4 ± 3.7**	75.23	0.30 ± 0.53*

Data are arranged as mean ± SEM ANOVA followed by Dunnet's *t* test where $^{*}p < 0.05$; $^{**}p < 0.01$.

of 69.52–85.71%, which was quite close to the standard drug diclofenac (90%). All these compounds possess electron withdrawing groups i.e. Cl and NO_2 in the quinoline ring. It was also observed that **5d** exhibited the most powerful analgesic activity by peripheral and central mechanism and it is a furanone derivative containing a naphthyl ring at position 5 in contrast to other three compounds that possess a biphenyl ring. Although, compound **6b**, a pyrrolone derivative, displayed moderate activity by tail immersion method it showed 67.14% protection in acetic acid induced writhing model.

3.5. Acute ulcerogenic test

Results of severity index (ulcerogenic activity) of selected eight compounds indicated better tolerability and safer gastrointestinal profile in contrast to the standard drug diclofenac. Compounds **5d** and **7h** appeared to be the least toxic (0.30 ± 0.31 and 0.30 ± 0.53) as compared to diclofenac (0.86 ± 0.28). The result indicates that compounds were less toxic in terms of ulcerogenicity as compared to standard NSAID (Table 6).

3.6. Docking studies

The results of docking against COXII (PDB3LN1) are listed in Table 7. Docking scores of almost all synthesized compound were greater than the internal ligand value. The docking scores of tested compounds range between −104.82 and −160.96. The maximum number of hydrogen bond interactions shown by tested compounds was 6 comparable to internal ligand interaction values i.e., 9. The compounds which showed maximum docking score values are **7h** (−160.96) (Fig. 3), **7f** (−138.27), and **7g** (−137.53) in comparison with internal ligand value (−86.29) while the compounds that showed maximum interaction with receptor residues are **5d** (6) (Fig. 4), **5h** (5) and **6d** (6). The binding mode of standard, **7h** and **5d** into the COX 2 is illustrated in Figs. 3–5. From the docking results (Table 7) for anti-inflammatory activity it was observed that *N*-benzyl pyrrolones showed maximum mol dock score in comparison with pyrrolones, it may be due to increase in hydrophobicity and due to substitution of hydrogen atom of pyrrolone with benzyl group. On comparing the *in vivo* and *in silico* activity result of synthesized compounds, it was observed that the

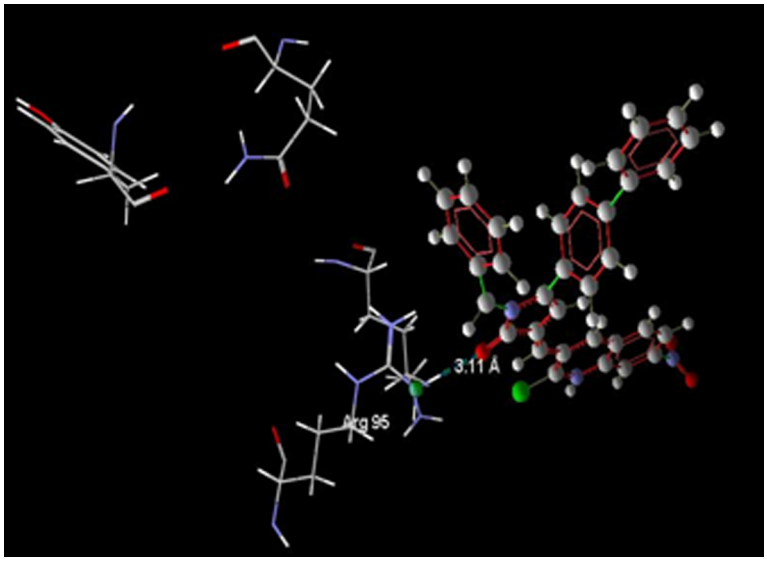


Figure 3 Binding mode of Compound **7h** into COX-2 (Maximum mol dock score = −160.96) shows only one hydrogen bond interaction between (=O of pyrrolone) and N(Arg 95) of distance 3.11 Å.

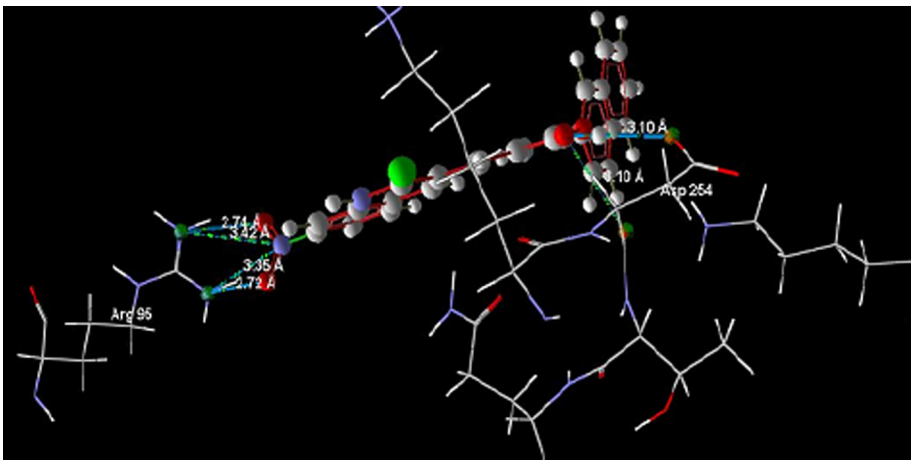


Figure 4 Binding mode of Compound **5d** into COX-2 (Maximum number of hydrogen bond interaction = 6). It has docking score −130.34 and forms 6 hydrogen bonds as shown by blue dotted lines showing 6 hydrogen bond interactions, two between N of NO_2 , one with N(Arg 95) of distance 3.42 Å other with N(Arg 95) of distance 3.35 Å, two with O of NO_2, both with N(Arg 95) of distance 2.74 Å and 2.72 Å, respectively, and other two between (=O, of furanone) with O(Asp 254) of distance 3.10 and 3.10 Å, respectively.

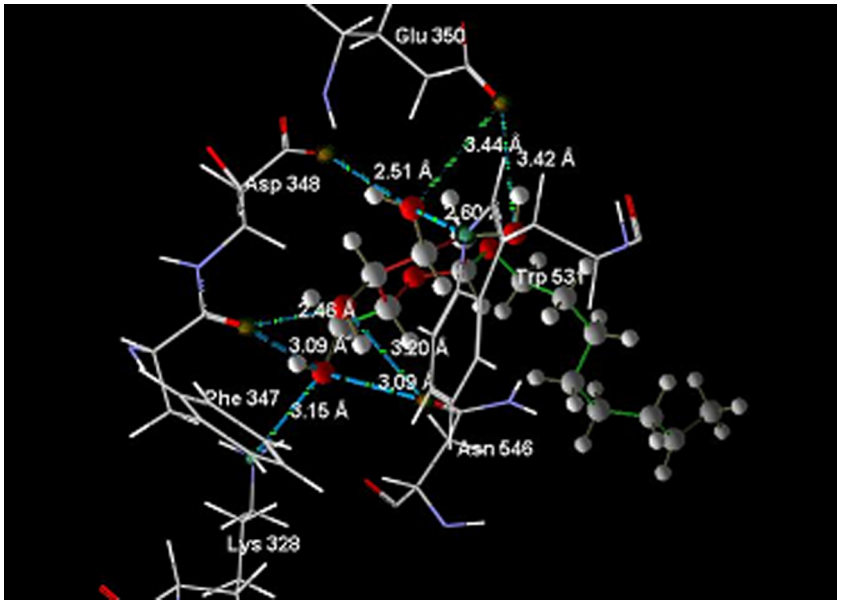


Figure 5 Binding mode of standard into COX-2 with Moldock score −86.29 and 9 hydrogen bond interactions, three between 4-OH of pyranose, one with N(Trp531) of distance 2.60 Å, second with O(Glu 31) of distance 2.51, third with O(Glu 350) of distance 3.44 Å, fourth between 3-OH of Pyranose and O(Glu 350) of distance 3.42 Å, two between 5-OH(Pyranose) and O(Phe 347) of distance 2.46 Å other with O(Asn 546) of distance 3.20 Å, three between O(CH_2OH), one with O(Phe 347) of distance 3.09 Å, other with N(Lys 328) and O(Asn 546) of distance 3.15 Å and 3.09 Å, respectively.

whole series of *N*-benzyl pyrrolones (**7a–h**) was most effective as anti-inflammatory agents as they exhibited maximum inhibition in edema volume and maximum mol dock score, respectively.

4. Conclusion

A total of 24 new quinoline derivatives containing five membered heterocyclic ring viz. furan-2(3*H*)-ones (**5a–h**), pyrrol-2(3*H*)-ones (**6a–h**) and *N*-benzyl-pyrrol-2(3*H*)-ones (**7a–h**) was designed and synthesized. *In vivo* biological testing results indicated some of the compounds to possess significant anti-inflammatory and analgesic activities with lesser GI toxicity. *N*-Benzyl-pyrrol-2(3*H*)-ones (**7a–h**) exhibited better anti-inflammatory activity than furan-2(3*H*)-ones and pyrrol-2(3*H*)-ones. Tested compounds also showed less GI toxicity and better tolerability than the standard drug diclofenac. Among the newer derivatives, four compounds **5d, 5h, 7f** and

Table 7 Docking studies of the synthesized compounds (**5a–h, 6a–h** and **7a–h**).

Compound no.	Mol dock score	No. of interaction	Ligand atom	PDB atom	Distance (Å)
Standard (PDB3LN2)	−86.29	9	O of 4-OH(pyranose)	N(Trp 531)	2.60
			O of 4-OH(pyranose)	O(Glu 31)	2.51
			O of 4-OH(pyranose)	O(Glu 350)	3.44
			3-OH	O(Glu 350)	3.42
			5-OH	O(Asn 546)	3.20
			5-OH	O(Phe 347)	2.46
			$O(CH_2OH)$	O(Phe 347)	3.09
			$O(CH_2OH)$	N(Lys 328)	3.15
			$O(CH_2OH)$	O(Asn 546)	3.09
5a	−110.78	2	O(furanone)	N(Lys 41)	3.38
				N(Cys 44)	2.76
5b	−131.18	3	=O(furanone)	N(Lys 239)	3.09
				O(Asp253)	2.54
				O(Asp 253)	3.08
5c	−104.82	3	=O(furanone)	O(Thr255)	3.29
				(Asp254)	3.21
				(Lys 229)	3.10
5d	−120.34	6	$N(NO_2)$	N(Arg 95)	3.41
			$O(NO_2)$	N(Arg95)	2.74
			$O(NO_2)$	N(Arg 95)	2.72
			$O(NO_2)$	N(Arg 95)	3.34
			$N(NO_2)$	O(Asp 254)	3.10
			=O(furanone)	O(Asp 254)	3.10
5e	−113.38	1	=O(furanone)	N(Lys 239)	2.61
5f	−112.40	3	O(furanone)	N(Lys 41)	3.34
			=O(furanone)	N(Thr 45)	3.55
			=O(furanone)	N(Cys 44)	2.67
			=O(furanone)	O(Asp 43)	3.33
5g	−113.65	3	O(furanone)	N(Lys 41)	3.26
			=O(furanone)	O(Asp 43)	3.42
			=O(furanone)	N(Cys44)	2.62
5h	−123.80	5	=O(furanone)	O(Asp 254)	2.86
			$O(NO_2)$	O(Asp 254)	2.95
			$O(NO_2)$	N(Arg 95)	2.68
			$O(NO_2)$	N(Arg 95)	3.82
				N(Arg 95)	3.19
6a	−113.41	2	N(Pyrrolone)	O(Cys 42)	3.26
			=O(Pyrrolone)	N(Cys 44)	2.73
6b	−118.08	3	N(Quinoline)	O(Glu 332)	3.14
			=O(Pyrrolone)	O(Ser 549)	2.67
			N(Pyrrolone)	O(Asp 254)	2.83
6c	−116.86	2	=O(Pyrrolone)	N(Cys 44)	2.77
				N(Cys 42)	3.32
6d	−109.29	6	$N(NO_2)$	N(Lys 328)	3.29
			$O(NO_2)$	N(Arg 95)	3.10
			$O(NO_2)$	N(Lys 328)	2.88
			N(Pyrrolone)	N(glu 332)	3.37
			N(Pyrrolone)	O(Asp 254)	3.51
			N(Pyrrolone)	O(Asp254)	2.72
			=O(Pyrrolone)	O(Ser 549)	3.05
6e	−113.15	1	=O(Pyrrolone)	N(Lys 239)	2.61
6f	−116.74	1	=O(Pyrrolone)	N(Lys 239)	2.60
6g	−117.22	1	=O(Pyrrolone)	N(Lys239)	2.61
6h	−115.98	1	=O(Pyrrolone)	N(lys 328)	3.48
7a	−129.12	2	N(Quinoline)	O(Lys 253)	3.56
			=O(Pyrrolone)	N(Lys 239)	3.25
7b	−126.69	1	N(Quinoline)	O(His 228)	3.32
7c	−128.274	1	N(Quinoline ring)	O(Ser 549)	2.98
7d	−134.12	1	$O(NO_2)$	N(Try531)	3.07
7e	−131.263	1	=O(Pyrrolone)	N(Lys 239)	2.60
7f	−138.27	1	=O(Pyrrolone)	N(Arg 95)	3.52
7g	−137.53	1	=O(Pyrrolone)	N(Arg 95)	3.53
7h	−160.96	1	=O(Pyrrolone)	N(Arg 95)	3.11

7h emerged as lead compounds. Further detailed studies are needed to confirm the potential of the furanone and *N*-benzyl-pyrrolone derivatives in anti-inflammatory therapy.

Acknowledgment

The authors are thankful to UGC, Govt. of India, New Delhi, for providing financial assistance.

References

Alam, M.M., Husain, A., Hasan, S.M., Suruchi, T., 2009. Synthesis and pharmacological evaluation of 2(3*H*)-furanones and 2(3*H*)-pyrrolones, combining analgesic and anti-inflammatory properties with reduced gastrointestinal toxicity and lipid peroxidation. Eur. J. Med. Chem. 44, 2636–2642.

Albrecht, A., Koszuk, J.F., Modranka, J., Rozalski, M., Krajewska, U., Janecka, A., Studzian, K., Janecki, T., 2008. Synthesis and cytotoxic activity of γ-aryl substituted α-alkylidene-γ-lactones and α-alkylidene-γ-lactams. Bioorg. Med. Chem. 16, 4872–4882.

Allison, M.C., Howatson, A.G., Torrance, C.J., Lee, F.D., Russell, R.I., 1992. Gastrointestinal damage associated with the use of non-steroidal anti-inflammatory drugs. N. Engl. J. Med. 327, 749–754.

Bailly, F., Queffelec, C., Mdemba, G., Mouscadet, J., Pommery, N., Pommery, J., Henichart, J., Cotelle, P., 2008. Synthesis and biological activities of a series of 4,5-diaryl-3-hydroxy-2(5*H*)-furanones. Eur. J. Med. Chem. 43, 1222–1229.

Bekhit, A.A., Abdel-Azeim, T., 2004. Design, synthesis and biological evaluation of some Pyrazole derivatives as anti-inflammatory-antimicrobial agents. Bioorg. Med. Chem. 12, 1935–1945.

Black, W.C., Brideau, C., Chan, C., Charleson, S., Cromlish, W., Gordon, R., Grimm, E.L., Hughes, G., Leger, S., Lii, C., Riendeau, D., Therien, M., Wang, Z., Xu, L., Prasit, P., 2003. 3,4-Diaryl-5-hydroxyfuranones: highly selective inhibitors of cyclooxygenase-2 with aqueous solubility. Bioorg. Med. Chem. Lett. 13, 1195–1198.

Cioli, V., Putzolu, S., Rossi, V., Barcellona, P.S., Corradino, C., 1979. The role of direct tissue contact in the production of gastro-intestinal ulcer by anti-inflammatory drugs in rats. Toxicol. Appl. Pharmacol. 50, 283–289.

Cruickshank, R., Duguid, JP., Marmion, BP., Swain, RHA., 1975. Medicinal Microbiology, 12th ed., vol. II, Churchill Livingstone, London, p. 196.

Flower, R.J., 2003. The development of COX-2 inhibitors. Nat. Rev. Drug Discov. 2, 179–191.

Harrak, Y., Rosell, G., Daidone, G., Plescia, S., Schillaci, D., Pujol, M.D., 2007. Synthesis and biological activity of new anti-inflammatory compounds containing the 1,4-benzodioxine and/or pyrrole system. Bioorg. Med. Chem. 15, 4876–4890.

Hashem, A.I., Abou-Elmagd, W.S.I., Abdalaziz, A., 2014. Synthesis and reactions of some 2(*3H*) and 2(*5H*) furanone derivatives: a comparative study. Eur. Chem. Bull. 3 (11), 1064–1068.

Hashem, A.I., Youssef, A.S., Kandeel, K.A., Abou-Elmagd, W.S., 2007. Conversion of some 2(3*H*)-furanones bearing a pyrazolyl group into other heterocyclic systems with a study of their antiviral activity. Eur. J. Med. Chem. 42, 934–939.

Husain, A., Hasan, S.M., Kumar, A., Alam, M.M., 2005. Synthesis and biological evaluation of 2-arylidene-4-(4-methoxyphenyl)but-3-en-4-olides. Asian J. Chem. 17 (3), 1579–1584.

Husain, A., Lal, S., Jyoti, 2013. Docking studies on butenolide derivatives as Cox-II inhibitors. Med. Chem. Res. 22 (11), 5536–5544.

Jashim Uddin, M., Rao, P., Knaus, E.K., 2004. Design and synthesis of acyclic triaryl (Z)-olefins: a novel class of cyclooxygenase-2 (COX-2) inhibitors. Bioorg. Med. Chem. 12, 5929–5940.

Khan, M.S.Y., Husain, A., 2002. Syntheses and reactions of some new 2-arylidene-4-(biphenyl-4-yl)-but-3-en-4-olides with a study of their biological activity. Pharmazie 57 (7), 448–452.

Lattmann, E., Dunn, S., Niamsanit, S., Sattayasai, N., 2005. Synthesis and antibacterial activities of 5-hydroxy-4-amino-2(5*H*)-furanones. Bioorg. Med. Chem. Lett. 15, 919–921.

Lattmann, E., Kinchington, M.D., Dunn, S., Singh, H., Ayuko, O.W., Tisdale, J.M., 2004. J. Pharm. Pharmacol. 56 (9), 1163–1170.

Leite, L., Jansone, D., Veveris, M., Cirule, H., Popelis, Y., Melikyan, G., Avetisyan, A., Lukevics, E., 1999. Vasodialating and antiarrhythmic activity of heteryl lactones. Eur. J. Med. Chem. 34, 859–865.

Levy, L.M., Cabrera, G.M., Wright, J.E., Seldes, A.M., 2003. 5*H*-Furan-2-ones from fungal cultures of *Aporpium caryae*. Phytochemistry 62, 239–243.

Mao, B., Geurts, K., Fananas-Mastral, M., Van Zij, A.W., Fletcher, S.P., Minnaard, A.J., Feringa, B.L., 2011. Catalytic enantioselective synthesis of naturally occurring butenolides via hetero-allylic alkylation and ring closing metathesis. Org. Lett. 13 (5), 948–951.

Moosavi-Movahedi, A.A., Hakimelahi, S., Chamani, J., Khodarahmi, G.A., Hassanzadeh, F., Luo, F.T., Ly, T.W., Shia, K.S., Yen, C.F., Jain, M.L., Kulatheeswaran, R., Xue, C., Pasdar, M., Hakimelahi, G.H., 2003. Design, synthesis, and anticancer activity of phosphonic acid diphosphate derivative of adenine-containing butenolide and its water-soluble derivatives of paclitaxel with high antitumor activity. Bioorg. Med. Chem. 11, 4303–4313.

Pohle, T., Brzozowski, T., Becker, J.C., Vander Voort, I.R., Markmann, A., Konturek, S.J., Moniczewski, A., Domschke, W., Konturek, J.W., 2001. Ascorbic acid enhances the inhibitory effect of aspirin on neuronal cyclooxygenase-2-mediated prostaglandin E2 production. Aliment Pharmacol. Ther. 15, 677–687.

Reyes, C.M., Kollman, P.A., 2000. Structure and Thermodynamics of RNA-protein binding: using molecular dynamics and free energy analyses to calculate the free energies of binding and conformational change. J. Mol. Biol. 297 (5), 1145–1158.

Rossi, R., Bellina, F., Biagetti, M., Mannina, L., 1998. Selective palladium-mediated synthesis of racemic 4,5-disubstituted 5*H*-furan-2-ones from 3-ynoic acids and organic halides. Tetrahedron Lett. 39 (41), 7599–7602.

Seigmund, E., Cadmus, R., Lu, G., 1957. A method for evaluating both non-narcotic and narcotic analgesics. Proc. Soc. Exp. Biol. 95, 729–731.

William, P.A., Lee, A., Blanchard, S., Teo, E., Deng, W., Tu, N., Tan, E., Sun, E., Goh, K.L., Ong, W.C., Ng, C.P., Goh, K.C., Bonday, Z.J., 2008. Structure-based design of Aurora A & B inhibitors. Comp. Aided Mol. Design 22 (12), 897–906.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. 111, 544–547.

Zarghi, P.N., Rao, P., Knaus, E.K., 2007. Synthesis and biological evaluation of methanesulfonamide analogues of rofecoxib: Replacement of methanesulfonyl by methanesulfonamido decreases cyclooxygenase-2 selectivity. Bioorg. Med. Chem. 15, 1056–1061.