Med Chem Res
DOI 10.1007/s00044-016-1732-6

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antimicrobial activity of novel quinoline derivatives bearing pyrazoline and pyridine analogues

Nisheeth C. Desai[1] · Bonny Y. Patel[1] · Bharti P. Dave[2]

Received: 4 May 2016 / Accepted: 11 October 2016

Abstract The present investigation is in the interest of some synthesized novel derivatives containing (5-(2-chloroquinolin-3-yl)-3-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanones (**4a–o**) moieties incorporated with different biological active heterocycles such as quinoline, pyrazoline and pyridine derivatives. For the determination of the compounds reported in this paper was based on IR, ^{1}H NMR, ^{13}C NMR and mass spectral data and same compounds were screened for their antibacterial and antifungal activity on four bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*) and three fungi (*Candida albicans*, *Aspergillus niger*, *Aspergillus clavatus*) using ampicillin and griseofulvin as the standard drugs. Cytotoxicity study was carried out using MTT colorimetric assay (HeLa cell line). Among the screened compounds, **4e**, **4f** and **4n** showed most potent antibacterial activity, while compounds **4d** and **4g** emerged as the most active against fungal strains. The results demonstrated that compound **4o** was remarkably active against all microbial strains. From the viewpoint of SAR studies, it was observed that the presence of electron withdrawing groups remarkably enhanced the antimicrobial activity of synthesized compounds. Additionally, preliminary MTT cytotoxicity studies on HeLa cells suggested that effective antimicrobial activity of **4e–g**, **4n** and **4o** was accompanied by low cytotoxicity.

Keywords Pyridine · Pyrazoline · 2-Chloroquinoline-3-carbaldehyde · Antimicrobial evaluation · Cytotoxicity

Abbreviations

Anti-HIV	Anti-human immunodeficiency virus
NCE'S	New chemical entity's
PDGF-RTK	Platelet-derived growth factor receptors tyrosine kinases
IR	Infrared
NMR	Nuclear magnetic resonance
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide

Introduction

A wide-ranging array of microbes including bacteria, viruses and fungi are becoming resistant to the standard marketed drugs used to treat infections. This resistance is a major hurdle for the treatment of highly infectious diseases globally. The World Health Organization (WHO) has considered this antimicrobial drug resistance and the dwindling number of active antimicrobial drugs to be one of the greatest threats to human health. Moreover, problems of multi-drug resistance of bacteria and fungi such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), Vancomycin resistant *enterococcus* (VRE) and Fluconazole

Electronic supplementary material The online version of this article (doi:10.1007/s00044-016-1732-6) contains supplementary material, which is available to authorized users.

✉ Nisheeth C. Desai
dnisheeth@rediffmail.com

[1] Division of Medicinal Chemistry, Department of Chemistry, (DST-FIST Sponsored & UGC NON-SAP), Mahatma Gandhi Campus, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar 364002, India

[2] Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar 364002, India

Published online: 19 October 2016

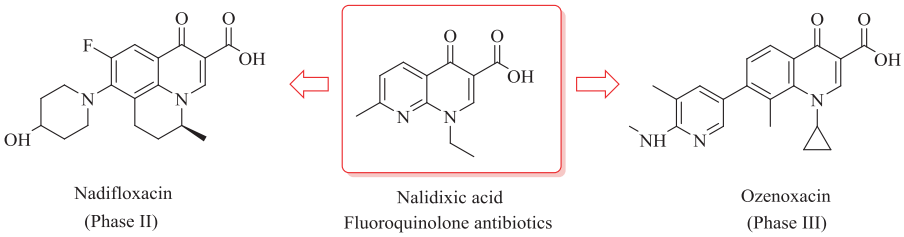


Fig. 1 Chemical remodeling of existing antibacterial drugs from potent molecules either in phase II or phase III of clinical trials

resistant *Candida* have reached an alarming level becoming a serious medical problem worldwide (Mishra et al. 2013; Cetinkaya et al. 2000; Gulshan and Moye-Rowley 2007). Therefore, it is necessary to develop antimicrobial agents with improved potency and the combat is still on to discover novel and potent antimicrobial agents for medicinal chemists (Moellering 2011). The structural modifications in existing drugs have shown astounding results in the field of drug discovery. Newly investigational drugs or trial drugs which utilize this strategy are safer with a broader spectrum of activity. Figure 1 represents potent molecules either in phase II or phase III of clinical trials from the existing NCE'S (Butler et al. 2013).

Searching for structure with significant bioactivity, we focused onto the development of molecules through combination of different active pharmacophores like quinolines, pyrazoline and pyridine into one core structure. That may lead to compounds with improved antimicrobial activity. Among the important heterocyclic moieties of biological and pharmacological attention, the quinoline ring is endowed with various activities such as antimicrobial (Desai et al. 2014), antiplasmodial (Vandekerckhove et al. 2015), antituberculosis (Keri and Patil 2014), anti-inflammatory (El-Feky et al. 2015), antimalarial (Vandekerckhove and D'hooghe 2015), anticancer (Spanò et al. 2015), antioxidant (Vivekanand et al. 2015), and anti-HIV (Ahmed et al. 2010). Furthermore, quinolines are used as an inhibitor in tyrokinase PDGF-RTK inhibiting agents (Maguire et al. 1994), SH2-containing inositol 5′-Phosphatase (SHIP) (Russo et al. 2015) and *Mycobacterium tuberculosis* DNA gyraseB inhibitors (Medapi et al. 2015).

2-Pyrazoline derivatives have been reported to exhibit various pharmacological activities such as antimicrobial (Karthikeyan et al. 2007), anti-inflammatory (Barsoum et al. 2006), antihypertensive, anti-tumor (Lin et al. 2007), anticancer (Gowramma et al. 2009) and anticonvulsant (Ozdemir et al. 2007). In addition, pyrazolines are also reported to possess cytotoxic properties against human lung tumor cell line (A549) (Greenlee et al. 2001). Nowadays some newly steroidal pyrazoline are also synthesized for finding a novel drug molecule (Shamsuzzaman and Mohd 2012). Pyridine is a versatile bioactive heterocycle having its wide presence in many synthetic drugs such as rosiglitazone (antidiabetic), pioglitazone (hypoglycemic), lansoprazole (proton-pump inhibitor), and etoricoxib (COX-2 selective inhibitor). The compounds containing pyridine scaffold exhibit anticancer (Kadayat et al. 2015), anti-inflammatory, anti-ulcer (Mohareb et al. 2015), anti-mycobacterial (Jose et al. 2015b) and antimicrobial (Jose et al. 2015a) activities.

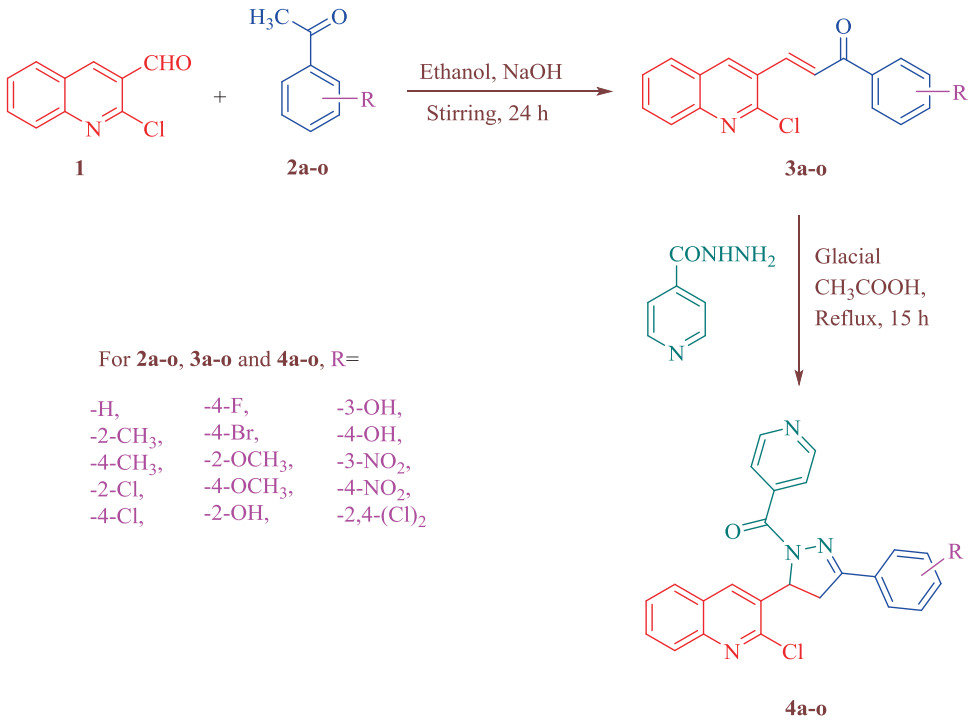
Prompted by the above-mentioned observations, we have focused onto construct some new antimicrobial derivatives bearing quinoline, pyrazoline and pyridine scaffolds and study the structure activity relationship due to substituent variations. Earlier, our research group had synthesized quinoline and other bioactive derivatives as potential antimicrobial agents (Desai et al. 2013a, b, 2015a, b). In continuation to this, (5-(2-chloroquinolin-3-yl)-3-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanones (**4a–o**) have been synthesized and screened for their antimicrobial activity. In addition, cytotoxicity studies were also conducted on HeLa cell lines to assess the ability of these compounds to inhibit the cell growth. The newly synthesized compounds (**4a–o**) were elucidated by IR, NMR and mass spectroscopy for their structural characterization.

Results and discussion

Chemistry

Synthetic strategies adopted to achieve the target compounds are depicted in Scheme 1. Here, scaffold **4** is a part of synthesis of new chemical entities in the form of antimicrobial agents. According to Scheme 1, the key chalcone derivatives 3-(2-chloroquinolin-3-yl)-1-(aryl)prop-2-en-1-ones (**3a–o**) are used as precursors for the synthesis of title compounds (**4a–o**). Compounds (**3a–o**) were synthesized through the Claisen–Schmidt condensation of equimolar amounts of different acetophenone derivatives (**2a–o**) and compound 2-chloroquinoline-3-carbaldehyde **1** by stirring the reactants in aqueous alcoholic solution containing sodium hydroxide at room temperature. Compound **1** is Vilsmeier–Haack reactions adduct; it provides a vital and efficient intermediate for the synthesis of several new substituted heterocyclic analogs. Chalcone compounds (**3a–o**) on cyclocondensation with isoniazid yielded final compounds (5-(2-chloroquinolin-3-yl)-3-aryl-4,5-dihydro-1*H*-

Scheme 1 Synthetic pathway of novel compounds **4a–o**



pyrazol-1-yl)(pyridin-4-yl)methanones (**4a–o**). Further it has been observed that compounds (**4a–o**) have pyrazoline core nucleus and were generated through cyclization, migration of α, β unsaturated double bonds, and diminishing of carbonyl group.

Designed series of molecules (**4a–o**) were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry techniques. IR spectrums of title compounds (**4a–o**) gave stretching vibrations at 3035–3059 cm^{-1} due to aromatic C–H stretching vibrations corresponds to methylene group that appears over the range at 2872–2926 cm^{-1}. The strong intensity absorption bands at 1645–1696 cm^{-1} is due to stretching vibrations of C=O group. The characteristic signals in ^{1}H NMR of compounds (**4a–o**) were of three pyrazoline protons which displayed doublet of doublet. Out of three, two pyrazoline protons (C-13) H_a and H_b give AB system. Part one of AB system displayed a signal at $\delta =$ 3.29–3.40 ppm as doublet of doublet with coupling constants 17.42–17.55 and 3.04–3.10 Hz and part two of AB system appeared as doublet of doublet at $\delta = 3.74$–3.82 ppm with coupling constants 17.40–17.52 and 11.01–11.16 Hz. Proton H_c of pyrazoline (C-11, methine proton) also appeared as doublet of doublet at $\delta = 6.02$–6.09 ppm with coupling constants in range of 11.08–11.22 and 3.08–3.17 Hz. Moreover, ^{13}C NMR confirmed the final compounds structure by the appearance of signals at $\delta = 171.2$–172.9 ppm due to carbonyl carbon of isoniazid directly attached to pyrazoline nitrogen. Compounds (**4a–o**) showed signal at $\delta = 39.1$–39.9 ppm corresponding to carbon of methylene group present in pyrazoline nucleus and methine carbon showed a chemical shift at $\delta =$ 60.0–66.6 ppm. Furthermore, the mass spectrum of (**4a–o**) showed a molecular ion peak corresponding to molecular formula (**4a–o**) along with of other fragment peaks, which supported the proposed structure of compounds. The detail discussion of characterization data are given in experimental section. The structure and carbon numbering of compound (**4a–o**) as an example are described in Fig. 2.

Discussion of antibacterial screening

Amongst synthesized compounds (**4a–o**), several compounds exhibited antimicrobial potency that diverged from good to excellent. On the basis of antibacterial screening results from Table 1, compound **4o** (-2,4-(Cl)$_2$) displayed good activity against *E. coli*, while compounds **4f** (-4-F) and **4n** (-4-NO$_2$) possessed very good activity against *E. coli* at 50 μg ml^{-1} MIC. It is noteworthy that compound **4e** (-4-Cl) showed highest inhibition at MIC = 12.5 μg ml^{-1} against the same Gram-negative bacterium, i.e. *E. coli*. In the case of *P. aeruginosa*, compound **4o** (-2,4-(Cl)$_2$) showed same inhibition as to the standard drug. While compounds **4e** (-4-Cl) and **4n** (-4-NO$_2$) inhibited bacteria at MIC = 50 μg ml^{-1} (very good activity) and maximum inhibition (MIC = 12.5 μg ml^{-1}) was shown by compound **4f** (-4-F) against *P. aeruginosa*. Moreover, compound **4e** (-4-Cl) displayed good activity and compounds **4f** (-4-F) and **4n** (-4-NO$_2$) showed very good activity at 250 and 100 μg ml^{-1} MIC

values against *S. aureus*. Compound **4o** (-2,4-$(Cl)_2$) exhibited inhibition at MIC = 12.5 μg ml^{-1} against Gram-positive bacterium, i.e. *S. aureus*. In case of Gram-negative bacterium (*S. pyogenes*), compounds **4e** (-4-Cl) and **4o** (-2,4-$(Cl)_2$) exhibited good activity at MIC = 50 μg ml^{-1} and compound **4f** (-4-F) displayed very good activity against *S. pyogenes*. Compound **4n** (-4-NO_2) had shown MIC 12.5 μg ml^{-1} which observed as the highest inhibition against *S. pyogenes*. The remaining compounds of the series possessed feeble antibacterial activity as shown in Table 1. Tabular data revealed that the presence of functional group at *para* position improved antibacterial activity as compared to *ortho* and *meta* substituted compounds.

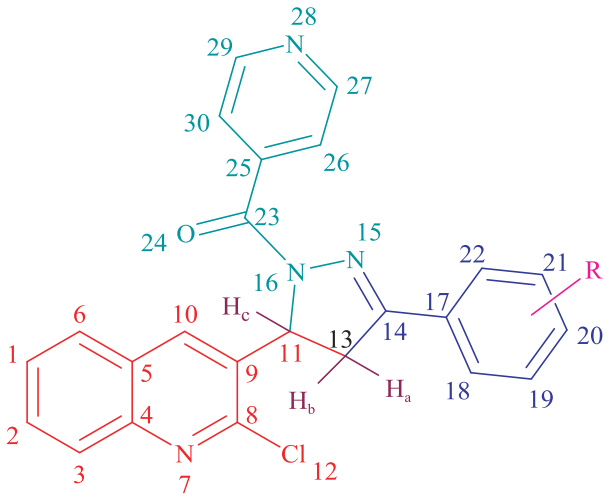


Fig. 2 Carbon numbering of the final compounds **4a–o**

Discussion of antifungal screening

Minimum inhibitory concentration (MIC) values of antifungal activity spotted against *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus* were by conventional broth micro dilution method. We have discussed and compared antifungal activities based on standard drug Griseofulvin shown in Table 1. Compounds **4m** (-3-NO_2), **4n** (-4-NO_2) and **4o** (-2,4-$(Cl)_2$) exhibited same MIC value as standard drug against *C. albicans*. Excellent and very good activity possessed against *C. albicans* by compounds **4d** (-2-Cl) and **4g** (-4-Br) at MIC = 50 and 100 μg ml^{-1}, respectively. Same compounds **4d** and **4g** also displayed very good activity against *A. niger*, while highest inhibition (12.5 μg ml^{-1}) exists in di-chloro substituted compounds, i.e. **4o** (-2,4-$(Cl)_2$). Furthermore, compounds **4g** and **4o** having

Table 1 Antimicrobial screening of the compounds **4a–o**

Sr. No.	-R	Minimum inhibitory concentrations						
		For bacteria (MIC) in μg ml^{-1}				For fungi (MIC) in μg ml^{-1}		
		Gram-negative		Gram-positive		Fungi		
		E.c.	P.a.	S.a.	S.p.	C.a.	A.n.	A.c.
4a	–H	250	500	500	500	1000	500	1000
4b	-2-CH_3	500	250	>1000	250	1000	500	250
4c	-4-CH_3	250	250	500	>1000	1000	250	500
4d	-2-Cl	100	100	250	250	**50**	**50**	**12.5**
4e	-4-Cl	**12.5**	**50**	**250**	**100**	>1000	250	250
4f	-4-F	**50**	**12.5**	**100**	**50**	1000	250	250
4g	-4-Br	250	250	250	100	**100**	**50**	**50**
4h	-2-OCH_3	500	1000	500	250	1000	500	250
4i	-4-OCH_3	>1000	500	1000	500	>1000	250	500
4j	-2-OH	250	250	1000	500	>1000	250	1000
4k	-3-OH	250	250	500	>1000	1000	>1000	500
4l	-4-OH	500	250	>1000	250	1000	500	250
4m	-3-NO_2	250	250	500	100	**500**	250	250
4n	-4-NO_2	**50**	**50**	**100**	**12.5**	**500**	250	1000
4o	-2,4-$(Cl)_2$	**100**	**100**	**12.5**	**100**	**500**	**12.5**	**50**
Ampicillin		100	100	250	100	–	–	–
Griseofulvin		–	–	–	–	500	100	100

E.c. *Escherichia coli* MTCC 443; P.a. *Pseudomonas aeruginosa* MTCC 1688; S.a. *Staphylococcus aureus* MTCC 96; S.p. *Staphylococcus pyogenes* MTCC 442; C.a. *Candida albicans* MTCC 227; A.n. *Aspergillus niger* MTCC 282; A.c. *Aspergillus clavatus* MTCC 1323

Bold values indicate the most active compounds

Table 2 Levels of cytotoxicity prompted by compounds **4a–o** on HeLa cells

Sr. No.	Cytotoxicity (IC_{50} μM)[a] HeLa[b]
4a	60.74
4b	81.14
4c	78.16
4d	87.53
4e	>100
4f	>100
4g	>100
4h	69.09
4i	58.74
4j	88.84
4k	66.34
4l	75.98
4m	58.65
4n	>100
4o	>100
Doxorubicin	3.24

[a] IC_{50} is the concentration required to inhibit 50 % of cell growth

[b] HeLa human cervical cancer cell line

-4-Br, -2,4-$(Cl)_2$ functional groups respectively, possessed very good activity against *A. clavatus*, while excellent activity against *A. clavatus* was demonstrated by -2-Cl (**4d**) derivative. Compound **4o** having electron withdrawing groups in structure proves its significance to almost all bacterial and fungal strains that makes a great imaginary impact in antimicrobial activity.

Discussion of in vitro cytotoxicity studies

In vitro cytotoxic activity of newly synthesized compounds (**4a–o**) was evaluated against human cervical cancer cell line (HeLa) by the MTT colorimetric assay (Mosmann 1983), which measures the reduction of tetrazolium bromide salt into a formazan dye by mitochondrial dehydrogenases in treated vs. untreated cells. The IC_{50} values achieved for these compounds were shown in Table 2. Cytotoxicity results displayed that the derivatives **4e** (4-Cl), **4f** (4-F), **4g** (4-Br), **4n** (4-NO_2) and **4o** (2,4-$(Cl)_2$) accounted no toxicity at concentration of 100 μg/ml ($IC_{50} > 100$ μg/ml), while other derivatives exhibited moderate toxicity against HeLa cell lines.

Experimental

Melting points were noted on Gallenkamp apparatus and were left uncorrected. The completion of reaction and the purity of all compounds was checked on aluminum-coated TLC plates 60, F_{245} (E. Merck) using various solvent systems as mobile phase and visualized under ultraviolet (UV) light, or iodine vapor. Perkin-Elmer 2400 CHN analyzer was used for elemental analysis (% C, H, N). IR spectra were recorded on Perkin Elmer FT-IR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker Avance II 400 MHz while ^{13}C NMR spectra on Varian Mercury-400, 100 MHz in $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard using 5 mm tube. Chemical shifts were reported in ppm units with use of δ scale. Mass spectra were also scanned on a Shimadzu LCMS 2010 spectrometer. The essential chemicals were purchased from E. Merck. Buchi Rota vapor instrument was used for distillation purpose.

Preparation of 2-chloroquinoline-3-carbaldehyde (1)

To a solution of acetanilide (5 mmol) in dry DMF (15 mmol) at 0–5 °C with stirring phosphoryl chloride ($POCl_3$) (60 mmol) was added drop-wise and the mixture stirred at 100 °C for 16–20 h. The mixture was poured into crushed ice, stirred for 5 min and the resulting solid filtered, washed well with water and dried. The yellow crystals formed were filtered, washed with water and crystallized using ethyl acetate (Meth-Cohn 1993). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.59 (t, 1H, Ar–H), 7.69 (t, 1H, Ar–H), 8.01 (d, 1H, Ar–H), 8.17 (d, 1H, Ar–H), 8.55 (s, 1H, Ar–H), 9.61 (s, 1H, Ar–H); anal. calcd. for $C_{10}H_6ClNO$: C, 62.68; H, 3.16; N, 7.31; found: C, 62.73; H, 3.24; N, 7.36.

General procedure of 3-(2-chloroquinolin-3-yl)-1-(aryl) prop-2-en-1-ones (3a–o)

Compounds 3-(2-chloroquinolin-3-yl)-1-(aryl)prop-2-en-1-ones (**3a–o**) were prepared according to the literature method (Elgazwy 2008). All other derivatives were prepared by the same method and monitored by thin layer chromatography (TLC). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.31–7.74 (m, 7H, Ar–H), 7.78 (d, 1H), 7.97 (d, 3H), 8.21 (s, 1H, quinoline-H_4); anal. calcd. for $C_{18}H_{12}ClNO$: C, 73.60; H, 4.12; N, 4.77; found: C, 73.69; H, 4.19; N, 4.70.

General procedure of (5-(2-chloroquinolin-3-yl)-3-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)(pyridin-4-yl)methanones (4a–o)

A mixture of differently substituted quinolinyl chalcones **3a–o** (0.01 mol) and isoniazid (0.02 mol) were taken in 20 ml glacial acetic acid and refluxed at 120 °C over a period of 8 h. The mixture was concentrated under vacuum and diluted with ice cold water. The separated solid was filtered, dried, and crystallized from ethanol (95 %).

*(5-(2-Chloroquinolin-3-yl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4a**)*

Yellow crystals; yield: 73 %; mp 118–120 °C; IR (KBr) ν_{max} 3064 (C–H, aromatic), 2871 (C–H stretching, –CH_2-group), 1692 (C=O stretching), 1579 (C=N), 1513 (C=C), 735 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.29$ (dd, $J_{ab} = 17.45$ Hz, $J_{ac} = 3.05$ Hz, 1H, H_a), 3.78 (dd, $J_{ab} = 17.41$ Hz, $J_{bc} = 11.02$ Hz, 1H, H_b), 6.06 (dd, $J_{ac} = 3.09$ Hz, $J_{bc} = 11.08$ Hz, 1H, H_c), 7.06–8.32 (m, 14H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.6$ (C-12, CH_2 of pyrazoline), 62.7 (C-11, CH–N of pyrazoline), 122.1–148.2 (Ar–C), 152.1 (C-8, C–Cl of quinoline), 156.6 (C-13, C=N of pyrazoline), 167.3 (C-23, C=O); LCMS: *m/z* 412.11 [M^+]; anal. calcd. for $C_{24}H_{17}ClN_4O$: C, 69.82; H, 4.15; N, 13.57; Found: C, 69.86; H, 4.14; N, 13.66.

*(5-(2-Chloroquinolin-3-yl)-3-(o-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4b**)*

Cream crystals; yield: 67 %; mp 184–185 °C; IR (KBr) ν_{max} 3065 (C–H, aromatic), 2922, 2875 (C–H stretching, –CH_3 group, –CH_2-group), 1690 (C=O stretching), 1584 (C=N), 1516 (C=C), 736 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 2.30$ (s, 3H, Ar–CH_3), 3.38 (dd, $J_{ab} = 17.44$ Hz, $J_{ac} = 3.07$ Hz, 1H, H_a), 3.74 (dd, $J_{ab} = 17.43$ Hz, $J_{bc} = 11.04$ Hz, 1H, H_b), 6.05 (dd, $J_{ac} = 3.10$ Hz, $J_{bc} = 11.09$ Hz, 1H, H_c), 7.10–8.38 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 20.1$ (C-15, Ar–CH_3), 43.3 (C-12, CH_2 of pyrazoline), 62.5 (C-11, CH–N of pyrazoline), 122.2–148.4 (Ar–C), 152.2 (C-8, C–Cl of quinoline), 156.7 (C-13, C=N of pyrazoline), 167.4 (C-23, C=O); LCMS: *m/z* 426.12 [M^+]; anal. calcd. for $C_{25}H_{19}ClN_4O$: C, 70.34; H, 4.49; N, 13.12; found: C, 70.42; H, 4.46; N, 13.20.

*(5-(2-Chloroquinolin-3-yl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4c**)*

Light cream crystals; yield: 65 %; mp 172–174 °C; IR (KBr) ν_{max} 3063 (C–H, aromatic), 2920, 2878 (C–H stretching, –CH_2-group, –CH_3 group), 1670 (C=O stretching), 1582 (C=N), 1518 (C=C), 734 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 2.25$ (s, 3H, Ar–CH_3), 3.30 (dd, $J_{ab} = 17.43$ Hz, $J_{ac} = 3.06$ Hz, 1H, H_a), 3.78 (dd, $J_{ab} = 17.43$ Hz, $J_{bc} = 11.03$ Hz, 1H, H_b), 6.04 (dd, $J_{ac} = 3.09$ Hz, $J_{bc} = 11.10$ Hz, 1H, H_c), 7.10–8.32 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 21.5$ (C-17, Ar–CH_3), 43.4 (C-12, CH_2 of pyrazoline), 62.7 (C-11, CH–N of pyrazoline), 122.0–148.6 (Ar–C), 152.1 (C-8, C–Cl of quinoline), 156.5 (C-13, C=N of pyrazoline), 167.2 (C-23, C=O); LCMS: *m/z* 426.12 [M^+]; anal. calcd. for $C_{25}H_{19}ClN_4O$: C, 70.34; H, 4.49; N, 13.12; found: C, 70.40; H, 4.42; N, 13.16.

*(3-(2-Chlorophenyl)-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4d**)*

Light yellow crystals; yield: 69 %; mp 164–166 °C; IR (KBr) ν_{max} 3066 (C–H, aromatic), 2900 (C–H stretching, –CH_2-group), 1690 (C=O stretching), 1585 (C=N), 1520 (C=C), 745 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.40$ (dd, $J_{ab} = 17.45$ Hz, $J_{ac} = 3.06$ Hz, 1H, H_a), 3.78 (dd, $J_{ab} = 17.43$ Hz, $J_{bc} = 11.06$ Hz, 1H, H_b), 6.08 (dd, $J_{ac} = 3.11$ Hz, $J_{bc} = 11.12$ Hz, 1H, H_c), 7.18–8.43 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.1$ (C-12, CH_2 of pyrazoline), 62.6 (C-11, CH–N of pyrazoline), 122.2–148.9 (Ar–C), 128.6 (C-15, Ar–C–Cl), 152.5 (C-8, C–Cl of quinoline), 156.3 (C-13, C=N of pyrazoline), 167.4 (C-23, C=O); LCMS: *m/z* 446.07 [M^+]; anal. calcd. for $C_{24}H_{16}Cl_2N_4O$: C, 64.44; H, 3.61; N, 12.53; found: C, 64.49; H, 3.62; N, 12.58.

*(3-(4-Chlorophenyl)-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4e**)*

Yellow crystals; yield: 67 %; mp 122–124 °C; IR (KBr) ν_{max} 3059 (C–H, aromatic), 2926 (C–H stretching, –CH_2-group), 1645 (C=O stretching), 1587 (C=N), 1568 (C=C), 750 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.33$ (dd, $J_{ab} = 17.46$ Hz, $J_{ac} = 3.05$ Hz, 1H, H_a), 3.77 (dd, $J_{ab} = 17.45$ Hz, $J_{bc} = 11.07$ Hz, 1H, H_b), 6.04 (dd, $J_{ac} = 3.10$ Hz, $J_{bc} = 11.13$ Hz, 1H, H_c), 7.22–8.42 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.3$ (C-12, CH_2 of pyrazoline), 62.7 (C-11, CH–N of pyrazoline), 122.1–148.8 (Ar–C), 136.2 (C-17, Ar–C–Cl), 152.2 (C-8, C–Cl of quinoline), 156.4 (C-13, C=N of pyrazoline), 167.2 (C-23, C=O); LCMS: *m/z* 446.07 [M^+]; anal. calcd. for $C_{24}H_{16}Cl_2N_4O$: C, 64.44; H, 3.61; N, 12.53; Found: C, 64.53; H, 3.69; N, 12.60.

*(5-(2-Chloroquinolin-3-yl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4f**)*

Dark yellow crystals; yield: 71 %; mp 150–152 °C; IR (KBr) ν_{max} 3060 (C–H, aromatic), 2874 (C–H stretching, –CH_2-group), 1695 (C=O stretching), 1584 (C=N), 1528 (C=C), 740 (C–Cl stretching), 1152 (C–F stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.40$ (dd, $J_{ab} = 17.48$ Hz, $J_{ac} = 3.08$ Hz, 1H, H_a), 3.81 (dd, $J_{ab} = 17.42$ Hz, $J_{bc} = 11.10$ Hz, 1H, H_b), 6.02 (dd, $J_{ac} = 3.12$ Hz, $J_{bc} = 11.20$ Hz, 1H, H_c), 7.13–8.30 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.6$ (C-12, CH_2 of pyrazoline), 62.6 (C-11, CH–N of pyrazoline), 121.9–147.5 (Ar–C), 152.3 (C-8, C–Cl of quinoline), 156.8 (C-13, C=N of pyrazoline), 164.5 (C-17, Ar–C–F), 167.8 (C-23, C=O); LCMS: *m/z* 430.10 [M^+]; anal. calcd. for $C_{24}H_{16}ClFN_4O$: C, 66.90; H, 3.74; N, 13.00; found: C, 64.86; H, 3.65; N, 13.10.

*(3-(4-Bromophenyl)-5-(2-chloroquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4g**)*

Gray crystals; yield: 65 %; mp 141–143 °C; IR (KBr) ν_{max} 3064 (C–H, aromatic), 2879 (C–H stretching, $-CH_2$-group), 1693 (C=O stretching), 1585 (C=N), 1530 (C=C), 734 (C–Cl stretching), 663 (C–Br stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.39$ (dd, $J_{ab} = 17.50$ Hz, $J_{ac} = 3.05$ Hz, 1H, H_a), 3.77 (dd, $J_{ab} = 17.48$ Hz, $J_{bc} = 11.08$ Hz, 1H, H_b), 6.08 (dd, $J_{ac} = 3.14$ Hz, $J_{bc} = 11.22$ Hz, 1H, H_c), 7.16–8.44 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.8$ (C-12, CH_2 of pyrazoline), 62.7 (C-11, CH–N of pyrazoline), 122.1–147.5 (Ar–C), 126.2 (C-17, Ar–C–Br), 152.6 (C-8, C–Cl of quinoline), 157.0 (C-13, C=N of pyrazoline), 167.5 (C-23, C=O); LCMS: *m/z* 490.02 [M^+]; anal. calcd. for $C_{24}H_{16}ClBrN_4O$: C, 58.62; H, 3.28; N, 11.39; found: C, 58.70; H, 3.19; N, 11.46.

*(5-(2-Chloroquinolin-3-yl)-3-(2-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4h**)*

Light brown crystals; yield: 62 %; mp 195–197 °C; IR (KBr) ν_{max} 3066 (C–H, aromatic), 2937, 2879 (C–H stretching, $-OCH_3$ group, $-CH_2$-group), 1692 (C=O stretching), 1584 (C=N), 1531 (C=C), 736 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.35$ (dd, $J_{ab} = 17.54$ Hz, $J_{ac} = 3.10$ Hz, 1H, H_a), 3.67 (s, 3H, $-OCH_3$), 3.80 (dd, $J_{ab} = 17.52$ Hz, $J_{bc} = 11.15$ Hz, 1H, H_b), 6.03 (dd, $J_{ac} = 3.17$ Hz, $J_{bc} = 11.20$ Hz, 1H, H_c), 7.17–8.48 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.9$ (C-12, CH_2 of pyrazoline), 56.2 (Ar–OCH_3), 62.5 (C-11, CH–N of pyrazoline), 122.0–147.7 (Ar–C), 152.8 (C-8, C–Cl of quinoline), 156.7 (C-13, C=N of pyrazoline), 167.7 (C-23, C=O); LCMS: *m/z* 442.12 [M^+]; anal. calcd. for $C_{25}H_{19}ClN_4O_2$: C, 67.80; H, 4.32; N, 12.65; found: C, 67.91; H, 4.41; N, 12.58.

*(5-(2-Chloroquinolin-3-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4i**)*

Brownish crystals; yield: 63 %; mp 166–168 °C; IR (KBr) ν_{max} 3064 (C–H, aromatic), 2942, 2877 (C–H stretching, $-OCH_3$ group, $-CH_2$-group), 1694 (C=O stretching), 1585 (C=N), 1528 (C=C), 732 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.34$ (dd, $J_{ab} = 17.50$ Hz, $J_{ac} = 3.08$ Hz, 1H, H_a), 3.61 (s, 3H, $-OCH_3$), 3.82 (dd, $J_{ab} = 17.48$ Hz, $J_{bc} = 11.12$ Hz, 1H, H_b), 6.08 (dd, $J_{ac} = 3.14$ Hz, $J_{bc} = 11.18$ Hz, 1H, H_c), 7.10–8.44 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.6$ (C-12, CH_2 of pyrazoline), 55.5 (Ar–OCH_3), 62.4 (C-11, CH–N of pyrazoline), 121.8–148.1 (Ar–C), 152.6 (C-8, C–Cl of quinoline), 156.6 (C-13, C=N of pyrazoline), 167.4 (C-23, C=O); LCMS: *m/z* 442.12 [M^+]; anal. calcd. for $C_{25}H_{19}ClN_4O_2$: C, 67.80; H, 4.32; N, 12.65; found: C, 67.83; H, 4.39; N, 12.62.

*(5-(2-Chloroquinolin-3-yl)-3-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4j**)*

Yellow crystals; yield: 62 %; mp 155–157 °C; IR (KBr) ν_{max} 3407 (–OH), 3062 (C–H, aromatic), 2875 (C–H stretching, $-CH_2$-group), 1695 (C=O stretching), 1586 (C=N), 1527 (C=C), 745 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.36$ (dd, $J_{ab} = 17.48$ Hz, $J_{ac} = 3.06$ Hz, 1H, H_a), 3.77 (dd, $J_{ab} = 17.44$ Hz, $J_{bc} = 11.07$ Hz, 1H, H_b), 6.07 (dd, $J_{ac} = 3.10$ Hz, $J_{bc} = 11.11$ Hz, 1H, H_c), 7.12–8.48 (m, 13H, Ar–H), 9.17 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.8$ (C-12, CH_2 of pyrazoline), 62.3 (C-11, CH–N of pyrazoline), 121.7–148.5 (Ar–C), 152.5 (C-8, C–Cl of quinoline), 156.4 (C-13, C=N of pyrazoline), 162.5 (C-15, Ar–C–OH), 167.2 (C-23, C=O); LCMS: *m/z* 428.10 [M^+]; anal. calcd. for $C_{24}H_{17}ClN_4O$: C, 67.21; H, 4.00; N, 13.06; found: C, 67.29; H, 4.08; N, 13.10.

*(5-(2-Chloroquinolin-3-yl)-3-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4k**)*

Dark yellow crystals; yield: 60 %; mp 219–221 °C; IR (KBr) ν_{max} 3410 (–OH), 3060 (C–H, aromatic), 2878 (C–H stretching, $-CH_2$-group), 1693 (C=O stretching), 1588 (C=N), 1524 (C=C), 740 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.37$ (dd, $J_{ab} = 17.44$ Hz, $J_{ac} = 3.05$ Hz, 1H, H_a), 3.75 (dd, $J_{ab} = 17.46$ Hz, $J_{bc} = 11.09$ Hz, 1H, H_b), 6.08 (dd, $J_{ac} = 3.11$ Hz, $J_{bc} = 11.13$ Hz, 1H, H_c), 7.14–8.49 (m, 13H, Ar–H), 9.07 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.6$ (C-12, CH_2 of pyrazoline), 62.2 (C-11, CH–N of pyrazoline), 121.5–148.8 (Ar–C), 152.6 (C-8, C–Cl of quinoline), 156.6 (C-13, C=N of pyrazoline), 160.2 (C-16, Ar–C–OH), 167.4 (C-23, C=O); LCMS: *m/z* 428.10 [M^+]; anal. calcd. for $C_{24}H_{17}ClN_4O$: C, 67.21; H, 4.00; N, 13.06; found: C, 67.18; H, 4.08; N, 13.03.

*(5-(2-Chloroquinolin-3-yl)-3-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4l**)*

Light yellow crystals; yield: 70 %; mp 227–229 °C; IR (KBr) ν_{max} 3415 (–OH), 3063 (C–H, aromatic), 2879 (C–H stretching, $-CH_2$-group), 1696 (C=O stretching), 1585 (C=N), 1520 (C=C), 736 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.40$ (dd, $J_{ab} = 17.46$ Hz, $J_{ac} = 3.06$ Hz, 1H, H_a), 3.77 (dd, $J_{ab} = 17.47$ Hz, $J_{bc} = 11.07$ Hz, 1H, H_b), 6.07 (dd, $J_{ac} = 3.13$ Hz, $J_{bc} = 11.12$ Hz, 1H, H_c), 7.20–8.47 (m, 13H, Ar–H), 9.14 (s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.8$ (C-12, CH_2 of pyrazoline),

62.3 (C-11, CH–N of pyrazoline), 121.4–148.7 (Ar–C), 152.5 (C-8, C–Cl of quinoline), 156.7 (C-13, C=N of pyrazoline), 161.1 (C-17, Ar–C–OH), 167.5 (C-23, C=O); LCMS: *m/z* 428.10 [M^+]; Anal. Calcd. for $C_{24}H_{17}ClN_4O$: C, 67.21; H, 4.00; N, 13.06; found: C, 67.29; H, 4.05; N, 13.12.

*(5-(2-Chloroquinolin-3-yl)-3-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4m**)*

Brown crystals; yield: 61 %; mp 221–223 °C; IR (KBr) ν_{max} 3065 (C–H, aromatic), 2877 (C–H stretching, $-CH_2$-group), 1694 (C=O stretching), 1589 (C=N), 1518 (C=C), 1486, 1353 (N–O asymmetric, symmetric stretching, $-NO_2$ group), 735 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.38$ (dd, $J_{ab} = 17.43$ Hz, $J_{ac} = 3.05$ Hz, 1H, H_a), 3.74 (dd, $J_{ab} = 17.49$ Hz, $J_{bc} = 11.06$ Hz, 1H, H_b), 6.07 (dd, $J_{ac} = 3.15$ Hz, $J_{bc} = 11.17$ Hz, 1H, H_c), 7.25–8.56 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.5$ (C-12, CH_2 of pyrazoline), 62.7 (C-11, CH–N of pyrazoline), 121.5–149.1 (Ar–C), 140.2 (C-15, Ar–C–NO_2), 152.8 (C-8, C–Cl of quinoline), 155.8 (C-13, C=N of pyrazoline), 167.2 (C-23, C=O); LCMS: *m/z* 457.09 [M^+]; anal. calcd. for $C_{24}H_{16}ClN_5O_3$: C, 62.96; H, 3.52; N, 15.30; found: C, 62.93; H, 3.48; N, 15.26.

*(5-(2-Chloroquinolin-3-yl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4n**)*

Orange yellow crystals; yield: 65 %; mp 211–213 °C; IR (KBr) ν_{max} 3063 (C–H, aromatic), 2876 (C–H stretching, $-CH_2$-group), 1693 (C=O stretching), 1592 (C=N), 1519 (C=C), 1481, 1348 (N–O asymmetric, symmetric stretching, $-NO_2$ group), 731 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.40$ (dd, $J_{ab} = 17.44$ Hz, $J_{ac} = 3.07$ Hz, 1H, H_a), 3.76 (dd, $J_{ab} = 17.42$ Hz, $J_{bc} = 11.08$ Hz, 1H, H_b), 6.07 (dd, $J_{ac} = 3.12$ Hz, $J_{bc} = 11.09$ Hz, 1H, H_c), 7.26–8.59 (m, 13H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.6$ (C-12, CH_2 of pyrazoline), 62.6 (C-11, CH–N of pyrazoline), 121.4–149.3 (Ar–C), 150.1 (C-17, Ar–C–NO_2), 152.7 (C-8, C–Cl of quinoline), 155.6 (C-13, C=N of pyrazoline), 167.4 (C-23, C=O); LCMS: *m/z* 457.09 [M^+]; anal. calcd. for $C_{24}H_{16}ClN_5O_3$: C, 62.96; H, 3.52; N, 15.30; found: C, 62.91; H, 3.48; N, 15.31.

*(5-(2-Chloroquinolin-3-yl)-3-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-4-yl)methanone (**4o**)*

White yellow crystals; yield: 68 %; mp 231–233 °C; IR (KBr) ν_{max} 3068 (C–H, aromatic), 2872 (C–H stretching, $-CH_2$-group), 1690 (C=O stretching), 1591 (C=N), 1523 (C=C), 734 (C–Cl stretching) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 3.37$ (dd, $J_{ab} = 17.49$ Hz, $J_{ac} = 3.09$ Hz, 1H, H_a), 3.79 (dd, $J_{ab} = 17.46$ Hz, $J_{bc} = 11.09$ Hz, 1H, H_b), 6.09 (dd, $J_{ac} = 3.15$ Hz, $J_{bc} = 11.12$ Hz, 1H, H_c), 7.16–8.48 (m, 12H, Ar–H); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 43.8$ (C-12, CH_2 of pyrazoline), 62.7 (C-11, CH–N of pyrazoline), 121.5–149.7 (Ar–C), 125.3, 127.8 (C-15, C-17, Ar–C–Cl), 152.7 (C-8, C–Cl of quinoline), 155.4 (C-13, C=N of pyrazoline), 167.6 (C-23, C=O); LCMS: *m/z* 480.03 [M^+]; anal. calcd. for $C_{24}H_{15}Cl_3N_4O$: C, 59.84; H, 3.14; N, 11.63; found: C, 62.89; H, 3.17; N, 11.67.

Biological assay

Antibacterial bioassay

The newly synthesized compounds **(4a–o)** were screened for their antibacterial activity against Gram positive [*Staphylococcus aureus* (MTCC-96), *Streptococcus pyogenes* (MTCC-442)], and Gram-negative bacteria [*Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-1688)] at different concentrations of 1000, 500, 200, 100, 50, 25, 12.5 μg ml^{-1}. Several of the newly synthesized compounds **(4a–o)** were found to exhibit moderate to excellent antimicrobial activity. Antibacterial activity was carried out as per National Committee for Clinical Laboratory Standards (NCCLS) protocol using serial broth dilution method and all the standard strains used for the antimicrobial activity were procured from Institute of Microbial Technology (IMTECH), Chandigarh. "The drugs which were found to be active in primary screening were similarly diluted to obtain 100, 50, 25 and 12.5 μg ml^{-1} concentrations for secondary screening to test in a second set of dilution against all microorganisms. Inoculum size for test strain was adjusted to 10^6 CFU/ml (Colony Forming Unit per milliliter) by likening the turbidity. Mueller–Hinton Broth was used as a nutrient medium to grow and dilute the synthesized compound suspension for test organisms. 2 % DMSO was used as a diluent/vehicle to acquire the desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Diluted 1000 μg/ml concentrated solution of synthesized compounds were used as stock solution. The control tube containing not any antibiotic was instantly subcultured [before inoculation] by spreading a loopful evenly over quarter of a plate of medium suitable for the growth of test organisms. The culture tubes were then incubated for 24 h at 37 °C, and the growth was observed visually and spectrophotometrically. After that, 10 μg ml^{-1} suspensions were further inoculated on appropriate media and development was noted after 24 and 48 h. The lowest concentration preventing appearance of turbidity was considered as minimum inhibitory concentration (MIC, μg ml^{-1}) i.e., the amount of growth from the control tube before incubation

(which represents the original inoculum) was compared. Solvent had no influence on strain growth and the result of this was greatly affected by the size of inoculum. DMSO and sterilized distilled water were used as negative control while 'ampicillin' antibiotic (1 U strength) was used as positive control. Standard drug 'ampicillin' was used in this study for evaluating antibacterial activity which showed 100, 100, 250 and 100 μg ml^{-1} MIC against *E. coli, P. aeruginosa, S. aureus, and S. pyogene,* respectively as shown in Table 1".

Antifungal bioassay

"The same newly synthesized compounds **(4a–o)** were screened for their antifungal activity against in six sets against *C. albicans* (MTCC-227), *A. niger* (MTCC-282) and *A. clavatus* (MTCC-1323) at various primary concentrations of 1000, 500 and 250 μg ml^{-1}. The primary screen active compounds were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25 and 12.5 μg ml^{-1} concentrations for secondary screening to test in a second set of dilution against all microorganisms. 'Griseofulvin' was used as a standard drug for antifungal activity, which showed 500, 100 and 100 μg ml^{-1} MIC against *C. albicans, A. niger* and *A. clavatus*, respectively (Table 1). For growth of fungi, in the present procedure, we have used Sabourauds dextrose broth at 28 °C in aerobic condition for 48 h. DMSO and sterilized distilled water used as negative controls while 'griseofulvin' (1 U strength) was used as a positive control".

MTT assay for cytotoxic activity

In vitro cytotoxicity activity of compounds **(4a–o)** was measured by means of the IC_{50} using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay process. "The IC_{50} determination was achieved according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations. All synthesized compounds were dissolved in 0.1 % DMSO with the stock concentration of 10 g/l and diluted with medium freshly before drug administration. Cell lines were sowed into 96-well plates at density of 8000 cells/ well. After seeding it for 24 h, each compound dilution was added in duplicate, and cultivation continued at 37 °C in a moistened atmosphere containing 10 % FBS, 1 % glutamine, and 50 μM/ml gentamicin sulfate in a 5 CO_2 and 95 % air. After 24 h, 20 μL MTT reagent at 5 mg/ml in PBS (filter sterilized, light protected and stored at 4 °C) per well was added, and after 4 h of incubation at 37 °C, MTT is changed to a blue formazan product by mitochondrial succinate dehydrogenase. This product was eluted from cells by addition of 150 ml of DMSO. The absorbance at 570 nm was determined by an ELISA using ELX800 micro plate spectrophotometer".

SAR studies

Structure–activity relationship (SAR) studies revealed that the antimicrobial activity in heterocyclic class of quinoline, pyrazoline and pyridine molecules depend on the nature of the peripheral substituents and their spatial relationship

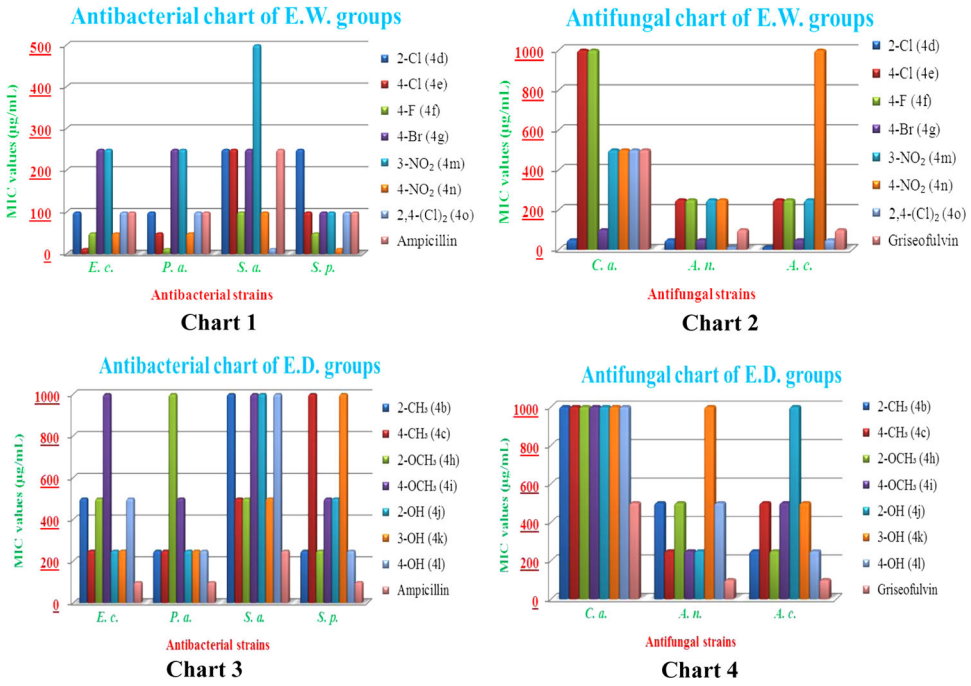


Fig. 3 Antibacterial and antifungal activity are expressed in the form of *bar chart* (*EW* electron withdrawing, *ED* electron donating)

within this skeleton. The pattern of substitution for the derivatives is carefully selected to confer different electronic environment of the molecules. The electronic nature of the substituent groups lead to significant discrepancy in antimicrobial activity. The presence of chloro, fluoro and nitro substituents at *para* position on aromatic ring system has amplified the antibacterial activity of compounds compared to those of electron donating substituents. By replacing substitution position to *ortho* and *meta*, compound tends to lost its potency (Chart **1** and **3**). Incorporation of electron donating groups such as methyl, methoxy and hydroxy diminished the antibacterial property (Chart **2** and **4**). The presence of lipophilic substituent on phenyl ring provides a positive effect on antifungal activity. In rationality with the above results, electron withdrawing groups like chloro and bromo on *ortho* and *para* substituted position showed optimal activity. It is concluded from Table 1 that, a compound without any substitution does not display antimicrobial activity against a panel of all microorganisms. Figure 3 showed antimicrobial activity in the form of bar chart.

Conclusion

We have synthesized compounds (**4a–o**) by conventional method with enlargement in yield of reactions. The synthesized compounds were screened for their in vitro antibacterial and antifungal activity against various bacterial and fungal strains. It is concluded from biological activity table that structural and electronic diversity of these products influences their biological activities. Compounds **4d**, **4e**, **4f**, **4g**, **4n** and **4o** are the most characteristic derivatives identified in present study because of their notable in vitro antimicrobial potency. SAR studies revealed that when electron withdrawing groups like fluoro, chloro, bromo and nitro present in structure demonstrated effective antimicrobial activity. Likewise di-substituted chloro group (compound **4o**) shows optimum inhibition over all strains of bacteria and fungi. It may be considered as a promising lead for further design and development of new lead molecules. As discussed from toxicity studies, we were encouraged to make modifications in electronic diversity on the basic structure of the final compounds (**4a–o**) for generation of non-toxic antimicrobial agents. It may be concluded that the presence of three different pharmacophore scaffolds enriched the biological activity.

Acknowledgments Authors are thankful to the University Grants Commission, New Delhi and Department of Science & Technology, New Delhi for financial support under the NON-SAP and DST-FIST programs respectively.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Ahmed N, Brahmbhatt KG, Sabde S, Mitra D, Singh IP, Bhutani KK (2010) Synthesis and anti-HIV activity of alkylated quinoline 2,4-diols. Bioorg Med Chem 18:2872–2879

Barsoum FF, Hosni HM, Girgis AS (2006) Novel bis(1-acyl-2-pyrazolines) of potential anti-inflammatory and molluscicidal properties. Bioorg Med Chem 14:3929–3937

Butler MS, Blaskovich MA, Cooper MA (2013) Antibiotics in the clinical pipeline in 2013. J Antibiot 66:571–591

Cetinkaya Y, Falk P, Mayhall CG (2000) Vancomycin-resistant enterococci. Clin Microbiol Rev 13:686–707

Desai NC, Bhatt N, Dodiya A, Karkar T, Patel B, Bhatt M (2015a) Synthesis, characterization and antimicrobial screening of thiazole based 1,3,4-oxadiazoles heterocycles. Res Chem Intermed. doi:10.1007/s11164-015-2196-x

Desai NC, Joshi VV, Rajpara KM, Vaghani HV, Satodiya HM (2013a) Synthesis of quinoline-pyrazoline based thiazole derivatives endowed with antimicrobial activity. Ind J Chem 52B:1191–1201

Desai NC, Kotadiya GM, Trivedi AR (2014) Studies on molecular properties prediction, antitubercular and antimicrobial activities of novel quinoline based pyrimidine motifs. Bioorg Med Chem Lett 24:3126–3130

Desai NC, Rajpara KM, Joshi VV, Vaghani HV, Satodiya HM (2013b) Synthesis of promising antimicrobial agents: a novel series of *N*-(4-(2,6-dichloroquinolin-3-yl)-6-(aryl)pyrimidin-2-yl)-2-morpholinoacetamides. Med Chem Res 22:1172–1183

Desai NC, Shihory N, Patel B, Bhatt M, Karkar T (2015b) Studies on antimicrobial evaluation of some 1-((1-(1*H*-Benzo[*d*]imidazol-2-yl)ethylidene)amino)-6-((arylidene)amino)-2-oxo-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitriles. Synth Commun 45:2701–2711

El-Feky SA, Abd El-Samii ZK, Osman NA, Lashine J, Kamel MA, Thabet HKh (2015) Synthesis, molecular docking and anti-inflammatory screening of novel quinoline incorporated pyrazole derivatives using the Pfitzinger reaction II. Bioorg Chem 58:104–116

Elgazwy ASH (2008) Synthesis and characterization of pyrido[1,2-*a*] quinoline palladacycles. Monatsh Chem 139:1285–1297

Gowramma B, Jubie S, Kaliranjan R, Gomathy S, Elango K (2009) Synthesis, anticancer activity of some 1-(Bis *N*, *N*-(Chloroethyl)-amino acetyl)-3, 5-disubstituted 1, 2-pyrazolines. Int J Pharma Tech Res 1:347–352

Greenlee RT, Hill-Harmon MB, Murray T, Thun M (2001) Cancer statistics, 2001. CA Cancer J Clin 51:15–36

Gulshan K, Moye-Rowley WS (2007) Multidrug resistance in Fungi. Eukaryot Cell 6:933–942

Jose G, Kumara THS, Nagendrappa G, Sowmya HBV, Jasinski JP, Millikan SP, More SS, Janardhan B, Harish BG, Chandrika N (2015a) Synthesis, crystal structure, molecular docking and antimicrobial evaluation of new pyrrolo [3, 2-*c*] pyridine derivatives. J Mol Struct 1081:85–95

Jose G, Kumara THS, Nagendrappa G, Sowmya HBV, Sriram D, Yogeeswari P, Sridevi JP, Guru Row TN, Hosamani AA, Ganapathy PSS, Chandrika N, Narendra LV (2015b) Synthesis, molecular docking and anti-mycobacterial evaluation of new imidazo[1,2-*a*]pyridine-2-carboxamidederivatives. Eur J Med Chem 89:616–627

Kadayat TM, Song C, Kwon Y, Lee ES (2015) Modified 2,4-diaryl-5*H*-indeno[1,2-*b*]pyridines with hydroxyl and chlorine moiety:

Synthesis, anticancer activity, and structure-activity relationship study. Bioorg Chem 62:30–40

Karthikeyan MS, Holla BS, Kumari NS (2007) Synthesis and antimicrobial studies on novel chloro-fluorine containing hydroxy pyrazolines. Eur J Med Chem 42:30–36

Keri RS, Patil SA (2014) Quinoline: a promising antitubercular target. Biomed Pharmacother 68:1161–1175

Lin R, Chiu G, Yu Y, Connolly PJ, Li S, Lu Y, Adams M, Fuentes-Pesquera AR, Emanuel SL, Greenberger LM (2007) Design, synthesis, and evaluation of 3,4-disubstituted pyrazole analogues as anti-tumor CDK inhibitors. Bioorg Med Chem Lett 17:4557–4561

Maguire MP, Sheets KR, McVety K, Spada AP, Zilberstein A (1994) A new series of PDGF receptor tyrosine kinase inhibitors: 3-substituted quinoline derivatives. J Med Chem 37:2129–2137

Medapi B, Renuka J, Saxena S, Sridevi JP, Medishetti R, Kulkarni P, Yogeeswari P, Sriram D (2015) Design and synthesis of novel quinoline-aminopiperidine hybrid analogues as Mycobacterium tuberculosis DNA gyraseB inhibitors. Bioorg Med Chem 23:2062–2078

Meth-Cohn O (1993) The synthesis of pyridines, quinolines and other related systems by the vilsmeier and the reverse vilsmeier method. Heterocycles 35:539–557

Mishra SK, Rijal PB, Pokhrel BM (2013) Emerging threat of multi-drug resistant bugs-*Acinetobacter calcoaceticus baumannii* complex and Methicillin resistant *Staphylococcus aureus*. BMC Res Notes 6:98–103

Moellering RC (2011) Discovering new antimicrobial agents. Int J Antimicrob Agents 37:2–9

Mohareb RM, Zaki MY, Abbas NS (2015) Synthesis, anti-inflammatory and anti-ulcer evaluations of thiazole, thiophene, pyridine and pyran derivatives derived from androstenedione. Steroids 98:80–91

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol 65:55–63

Ozdemir Z, Kandilci HB, Gümüşel B, Caliş U, Bilgin AA (2007) Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. Eur J Med Chem 42:373–379

Russo CM, Adhikari AA, Wallach DR, Fernandes S, Balch AN, Kerr WG, Chisholm JD (2015) Synthesis and initial evaluation of quinoline-based inhibitors of the SH2-containing inositol 5'-Phosphatase (SHIP). Bioorg Med Chem Lett 25:5344–5348

Shamsuzzaman A, Mohd G (2012) Facile synthesis of some new steroidal pyrazoline and 1′,3′,4′-oxadiazole. Ind J Chem 51B:760–764

Spanò V, Parrino B, Carbone A, Montalbano A, Salvador A, Brun P, Vedaldi D, Diana P, Cirrincione G, Barraja P (2015) Pyrazolo [3,4-*h*]quinolines promising photosensitizing agents in the treatment of cancer. Eur J Med Chem 102:334–351

Vandekerckhove S, D'hooghe M (2015) Quinoline-based antimalarial hybrid compounds. Bioorg Med Chem 23:5098–5119

Vandekerckhove S, Herreweghe SV, Willems J, Danneels B, Desmet T, de Kock C, Smith PJ, Chibale K, D'hooghe M (2015) Synthesis of functionalized 3-, 5-, 6- and 8-aminoquinolines via intermediate (3-pyrrolin-1-yl)- and (2-oxopyrrolidin-1-yl)quinolines and evaluation of their antiplasmodial and antifungal activity. Eur J Med Chem 92:91–102

Vivekanand B, Raj KM, Mruthyunjayaswamy BHM (2015) Synthesis, characterization, antimicrobial, DNA-cleavage and antioxidant activities of 3-((5-chloro-2-phenyl-1*H*-indol-3-ylimino)methyl) quinoline-2(1H)-thione and its metal complexes. J Mol Struct 1079:214–224