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Synthesis, characterization and in vitro screening on bacterial, fungal and malarial strain of piprazinyl cyano biphenyl based compounds

Mahesh H. Malani, Bharat Z. Dholakiya *

Department of Applied Chemistry, S.V. National Institute of Technology, Ichchhanath, Surat 395007, Gujarat, India

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ABSTRACT

A series of eight 4′-[4-(3-substituted phenyl-acryloyl)-piprazin-1-ylmethyl]-biphenyl-2-carbonitrile were synthesized using 4′-Bromomethyl-biphenyl-2-carbonitrile and 4-Acetyl piprazine as a starting material. Furthermore, there has been some additional work done investigating effects of these derivatives on biological activities on bacterial, fungal and malarial strain. Synthesized compounds were characterized using FTIR, ^{1}H NMR and ^{13}C NMR spectrometry. These compounds shows good antimalarial, antibacterial and antifungal activity. In fact some compounds are more potent than standard drug quinine and Ampicillin some are with comparable activity with Ampicillin and quinine.

1. Introduction

World Health Organization (WHO) accorded since 2008 estimative 247 million malaria cases among more than 3 billion people at risk, causing nearly one million deaths mostly of children under 5 years and pregnant women.[1] Malaria is a life-threatening parasitic disease caused, by protozoan parasites of the genus Plasmodium. Globally four species of the Plasmodium viz. *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* are responsible for human malaria and one another species *P. knowlesi* infecting humans have been documented recently [2,3]. Over the years chloroquine (CQ) has remained as the drug of choice for the malaria chemotherapy [4,5]. Among four species of human malaria parasite, *Plasmodium falciparum* is considered as most fetal due to the high mortality rate particularly in children. Several drugs are being used in malaria-endemic regions of the world to control, treat, and prevent malaria like, chloroquine, primaquine, sulfadoxine, pyrimethamine, etc. [6].

The piperazine ring nucleus is found in number of biologically active compounds, including several marketed drugs [7,8], and considered it as privileged structure in drug discovery [9]. For an exploratory medicinal chemistry program, we were interested in preparing a diverse set of tri substituted piperazine by using flexible synthesis route which will allow introduction of a variety of linkers and aryl groups. An orthogonal protection strategically approach using two piperazine nitrogen would facilitate the selective incorporation of active groups [10]. Readily available piperazine derivatives in choice as building blocks resulting in novel chemical entities with pharmacologically useful properties [11].

Utility of currently used antibiotics compromised by the emergence and subsequent spread of resistant pathogens, continuously in need for the development of novel antibacterial agents with broader spectrum of activity able to combat resistance [12]. Development of new antibacterial agents with novel structure and mode of action remains the prominent goal of scientists to solve increasing bacterial resistance gained by microorganism to classical antibacterial agents [13]. Fungi widely distributed and frequently appear in nature as pathogens in the animal and plant kingdoms [14]. Elemental sulfur has long been known to act as an antifungal agent. Tolnaftate, known antifungal agents has sulfur in an organically combined form e.g. *Allium sativum* (garlic) which is known to inhibit *Candida albicans* [15]. Kostanecki, who had done pioneer work in the synthesis of natural coloring compounds, first coined the term 'chalcone'. An interesting feature of chalcones is that they serve as starting materials for another class of naturally occurring and widely distributed pigments, flavones [16]. They are considered as precursors of flavonoids and isoflavonoids, which are abundant in edible plants. Chalcones are intermediates in the synthesis of flavones. Chemically they are open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system [17]. Chalcones consist of two aromatic

Abbreviations: AIBN, azobis isobutyronitrile; DDH, 1,3-dibromo-5,5-dimethyl-hydantoin; MTCC, microbial type culture collection; DMSO, di methyl sulphoxide; TEA, triethyl amine; DMF, dimethyl formamide; FTIR, Fourier transform infrared spectroscopy; NMR, nuclear magnetic resonance; TMS, tetramethylsilane; TLC, thin layer chromatography; MDC, dichloro methane; RPMI, Roswell Park Memorial Institute; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; RBCs, red blood cell; JSB, Jaswant–Singh–Bhattacherji.

* Corresponding author. Tel.: +91 9426473318, +91 9428949595; fax: +91 0261 227334.

E-mail addresses: maheshmalani.svnit@gmail.com (M.H. Malani), bharat281173@gmail.com (B.Z. Dholakiya).

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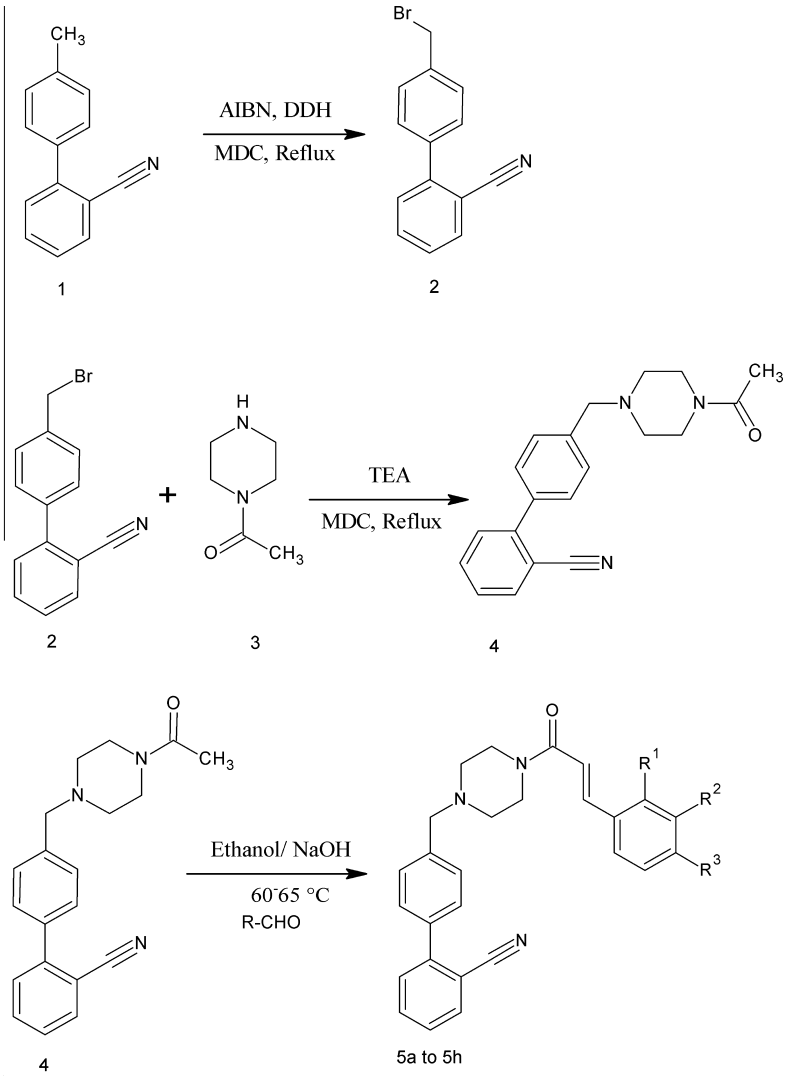

Scheme 1. Reaction scheme for synthesized compound.

rings trans configured and identified by three carbons system, of which two are connected by a double bond while the third is a carbonyl group [18]. Chalcones are readily synthesized by the base-catalyzed Claisen-Schimdt condensation of an aldehyde and appropriate ketone in a polar solvent like methanol or ethanol [19]. Chalcone derivatives have various therapeutic values including antioncogenic, antiinflammatory, analgesic, antiulcerative, antiviral, antibacterial, antifungal, and antimalarial properties [20].

In this paper we reported a synthesis of bioactive compounds (5a–5h) from 4′ methylbiphenyl-2-carbonitrile using alkali catalyst and alcohol as a solvent. All synthesized compounds were screened for bioactive assay.

2. Experimental

2.1. Materials

4′-(Bromomethyl) biphenyl-2-carbonitrile was prepared as per reported process [21] and 4-Acetyl piperazine, DBDMH, AIBN were purchased from Aldrich. The reactions were monitored and Rf value was determined using Merck Silica gel 60 and F-254 pre coated TLC plates (0.25-mm thickness). A spot on the TLC plates were visualized using ultraviolet light (254 nm). Reagents were of analytical reagent grade and were used without further purification. Solvents employed were purified by standard procedure.

2.2. Synthesis of 4′-(bromomethyl) biphenyl-2-carbonitrile 2

4′-(Bromomethyl) biphenyl-2-carbonitrile (Scheme 1) was prepared in present work using reported technique [21]. A mixture of 4′ methylbiphenyl-2-carbonitrile (1) 5.0 g, (0.0416 moles), DBDMH 8.08 g, (0.0282 moles), AIBN 0.12 g, (0.00079 moles), and dichloromethane (25 ml) as a solvent was charged in three neck flat bottom reaction flask. Reaction mass was continuously stirred and initially temperature was raised to 28–30 °C and then maintain to reflux for 8–9 h. After completion of the reaction, mass was cool to 20–22 °C and 10 ml water was added, stirred and transfer in separating funnel. Lower organic layer from separating funnel was washed with sodium bicarbonate solution followed by wash with water, and treated with sodium sulfate. Organic layer was evaporated to dryness and cremish colored solid having m.p. 125–127 °C (6.0 g 85% yield) was obtained.

2.3. Preparation of 4′-[(4-acetylpiperazin-1-yl)methyl]biphenyl-2-carbonitrile 4

4′-[(4-Acetylpiperazin-1-yl)methyl]biphenyl-2-carbonitrile (Scheme 1) was prepared as per follow. A mixture of 4′-(bromomethyl) biphenyl-2-carbonitrile 5.0 g, (0.0183 moles), 4-Acetyl piperazine (3) 2.35 g, (0.0183 mol), TEA 1.85 g, (0.0183 moles) and dichloromethane (30 ml) as a solvent was added in three neck

Table 1
Physico chemical papameter of synthesized compounds.

Sr. no.	Compound code	R_1, R_2 and R_3	% Yield	Color (solid powder)	Melting point
1	5a	R_1 = H, R_2 = H and R_3 = —OCH_3	85%	Cremish to off white	Dec.>250 °C
2	5b	R_1 = H, R_2 = —NO_2 and R_3 = H	79%	Cremish	200–201 °C
3	5c	R_1 = H, R_2 = H and R_3 = —NO_2	73%	Dark brown	195–197 °C
4	5d	R_1 = H, R_2 = H and R_3 = —CH_3	68%	Yellow	168–170 °C
5	5e	R_1 = H, R_2 = H and R_3 = —OH	83%	Light brown	>250 °C
6	5f	R_1 = Cl, R_2 = H and R_3 = H	67%	Dark yellow	85–87 °C
7	5g	R_1 = H, R_2 = H and R_3 = —Cl	69%	Light yellow	188–189°c
8	5h	R_1 = H, R_2 = H and R_3 = H (Naphthalene ring)	74%	Dark yellow	127–128 °C

flat bottom reaction flask with stirring at 28–30 °C. Gradually temperature was raised to reflux and maintain it for 3.0 h. Progress of the reaction was examined by TLC. After completion of reaction mass was evaporated to dryness to obtain oily sticky mass. 25 ml water and 25 ml ethyl acetate was charged in flask containing oily sticky mass and stirred it for 10 min at 25–30 °C. Reaction mass was transfer from reaction flask to separating funnel to obtain two layers. Top organic layer was treated with sodium sulfate and evaporated to dryness. Light yellow solid mass was obtain having melting point 90–95 °C (5.2 g, 88% yield).

2.4. Preparation of 4′-4-[3-(substituted-phenyl)-acryloyl]-piperazin-1-ylmethyl-biphenyl-2-carbonitrile compounds 5a-5 h

4′-4-[3-(Substituted-phenyl)-acryloyl]-piperazin-1-ylmethyl-biphenyl-2-carbonitrile (5a–5h) compounds were prepared from compound-4 and various aldehydes under alkaline conditions in present of alcohol. Reaction mass was continuously stirred and temperature was maintain at 55–60 °C for 12 h. Final targeted compounds were isolated from alcohol by using water at pH 6–7. Purification of these compounds were carried out by recrystallization in present of ethanol. All compounds were characterized by FTIR, ^{1}HNMR, ^{13}C NMR and screened for their biological activity viz, bacterial, fungal and malarial.

2.4.1. 4′-4-[3-(4-Methoxy-phenyl)-acryloyl]-piperazin-1-ylmethyl-biphenyl-2-carbonitrile 5a

Yield 85%; m.p. >250 °C; ^{1}H NMR (400 MHz, DMSO), 2.55 δ ppm (1H, d, *J* = 13.3 Hz, —CH═CH), 3.52 δ ppm (1H, m, —CH_2CHOHCH_2), 3.35 δ ppm (4H,dd, *J* = 5.2 Hz, Piprazine), 3.54 δ ppm (1H, d, *J* = 13.4 Hz, —CH═CH), 4.1 δ ppm (3H, s,Ar-OCH_3) 7.17–7.75 δ ppm (8H, m, Ar—H), 8.14 δ ppm (2H, s, —Ar—CH_2); ^{13}C NMR (100 MHz, DMSO) 22, 80, 126, 130, 132, 134, 142, 144, 190 δ ppm; FTIR (KBr) υ_{max} cm^{-1}: 2918 (C═C), 1650 (C═O), 2234 (C—N, Nitrile), 862 (p-disubstituted Ar).

2.4.2. 4′-4-[3-(3-Nitro-phenyl)-acryloyl]-piperazin-1-ylmethyl-biphenyl-2-carbonitrile 5b

Yield 79%; m.p. 200–201 °C; ^{1}H NMR (400 MHz, DMSO), 2.51 δ ppm (1H, d, *J* = 13.1 Hz, —CH═CH), 3.53 δ ppm (1H, m, —CH_2-CHOHCH_2), 3.39 δ ppm (4H,dd, *J* = 5.1 Hz, Piprazine), 3.57 δ ppm (1H, d, *J* = 13.2 Hz, —CH═CH), 7.20–7.76 δ ppm (8H, m, Ar—H), 8.16 δ ppm(2H, s, —Ar—CH_2); ^{13}C NMR (100 MHz, DMSO) 23, 79, 127, 129, 131, 133, 143, 145, 192 δ ppm; FTIR (KBr) υ_{max} cm^{-1}: 2916 (C═C), 1648 (C═O), 2226 (C—N, Nitrile), 780 (m-disubstituted Ar).

2.4.3. 4′-4-[3-(4-Nitro-phenyl)-acryloyl]-piperazin-1-ylmethyl-biphenyl-2-carbonitrile 5c

Yield 73%; m.p. 195–197 °C; ^{1}H NMR (400 MHz, DMSO), 2.52 δ ppm (1H, d, *J* = 13.2 Hz, —CH═CH), 3.55 δ ppm (1H, m, —CH_2-CHOHCH_2), 3.38 δ ppm (4H,dd, *J* = 5.2 Hz, Piprazine), 3.53 δ ppm (1H, d, *J* = 13.1 Hz, —CH═CH), 7.16–7.71 δ ppm (8H, m, Ar—H), 8.12 δ ppm(2H, s, —Ar—CH_2); ^{13}C NMR (100 MHz, DMSO) 20, 77, 123, 126, 127, 129, 138, 140, 186 δ ppm; FTIR (KBr) υ_{max} cm^{-1}: 2912 (C═C), 1644 (C═O), 2222 (C—N, Nitrile), 840 (p-disubstituted Ar).

2.4.4. 4′-[4-(3-p-Tolyl-acryloyl)-piperazin-1-ylmethyl]-biphenyl-2-carbonitrile 5d

Yield 68%; m.p. 168–170 °C; ^{1}H NMR (400 MHz, DMSO), 2.37 δ ppm (3H, s, Ar—CH_3), 2.53 δ ppm (1H, d, *J* = 13.3 Hz, —CH═CH), 3.37 δ ppm (4H,dd, *J* = 5.2 Hz, Piprazine), 3.55 δ ppm (1H, d, *J* = 13.2 Hz, —CH═CH), 7.18–7.73 δ ppm (8H, m, Ar—H), 8.15 δ

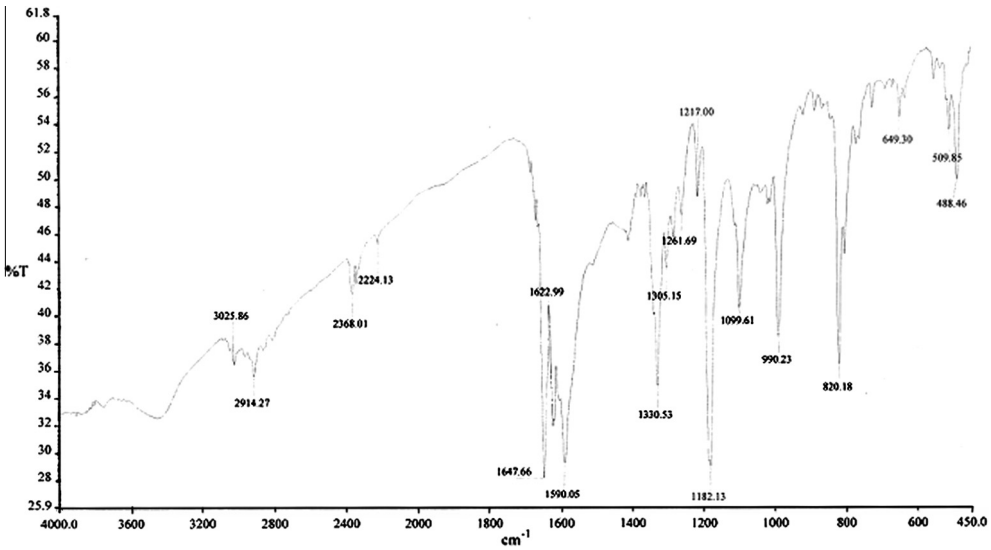

Fig. 1. IR Spectrum of synthesized compound.

Table 2
IR data of synthesized compounds.

Compounds	ν(—OH) cm^{-1}	ν(C=C) cm^{-1} (aliphatic)	ν(C=O) cm^{-1}	ν(—CN) cm^{-1} nitriles	ν(P-substitution aromatic ring) cm^{-1}	—NO_2 aromatic cm^{-1}
5a		2932(m)	1660(s)	2220(m)	835(m)	
5b		2942(m)	1686(s)	2232(m)	826(m)	1550(m)
5c		2954(m)	1674(s)	2228(m)	813(m)	1534(m)
5d		2914(m)	1647(s)	2224(m)	820(m)	
5e	3315(w)	2980(m)	1640(s)	2218(m)	833(m)	
5f		2955(m)	1700(s)	2229(m)	836(m)	
5g		2958(m)	1695(s)	2227(m)	816(m)	
5h		2970(m)	1710(s)	2238(m)	837(m)	

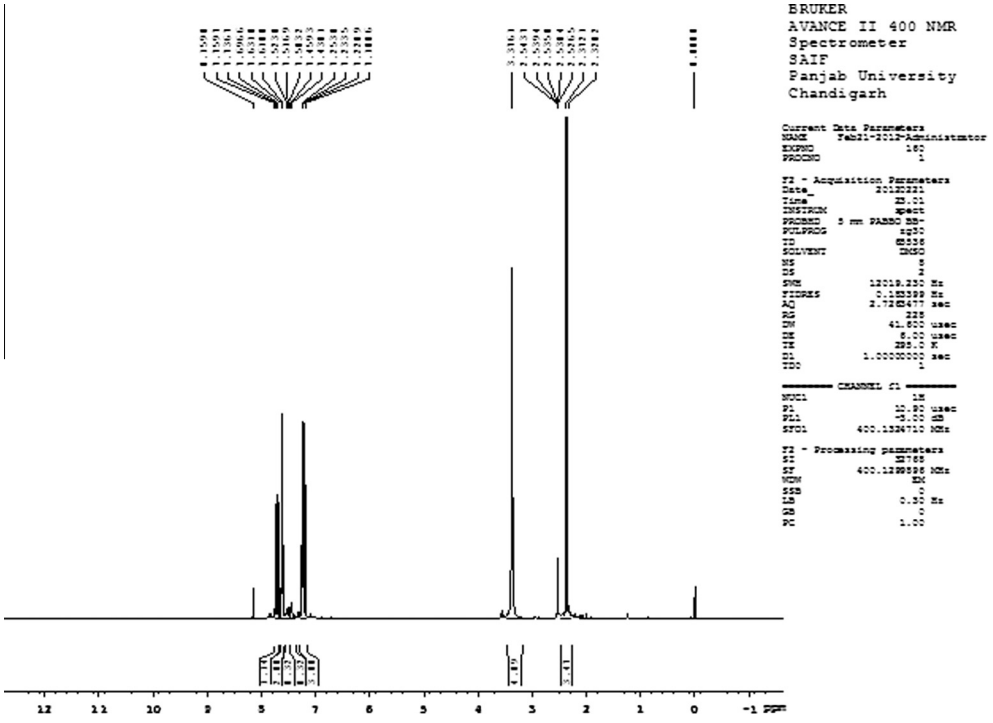

Fig. 2. H^1 NMR Spectrum of synthesized compound.

ppm(2H, s, —Ar—CH_2); ^{13}C NMR (100 MHz, DMSO) 21, 78, 124, 128, 129, 131, 140, 142, 188 δ ppm; FTIR (KBr) υ_{max} cm^{-1}: 2914 (C=C), 1647 (C=O), 2224 (C—N, Nitrile), 835 (p-disubstituted Ar).

2.4.5. 4′-4-[3-(4-Hydroxy-phenyl)-acryloyl]-piperazin-1-ylmethyl-biphenyl-2-carbonitrile 5e

Yield 83%; m.p. > 250 °C; ^{1}H NMR (400 MHz, DMSO), 2.55 δ ppm (1H, d, *J* = 13.4 Hz, —CH=CH), 3.52 δ ppm (1H, m, —CH_2CHOHCH_2), 3.40 δ ppm (4H,dd, *J* = 5.4 Hz, Piprazine), 3.59 δ ppm (1H, d, *J* = 13.3 Hz, —CH=CH), 7.21–7.76 δ ppm (8H, m, Ar—H), 8.16 δ ppm(2H, s, —Ar—CH_2), 8.8 δ ppm (1H, s, Ar—OH)^{13}C NMR (100 MHz, DMSO) 23, 81, 127, 131, 133, 135, 144, 147, 192 δ ppm; FTIR (KBr) υ_{max} cm^{-1}: 2916 (C=C), 1650 (C=O), 2228 (C—N, Nitrile), 826 (p-disubstituted Ar).

2.4.6. 4′-4-[3-(2-Chloro-phenyl)-acryloyl]-piperazin-1-ylmethyl-biphenyl-2-carbonitrile 5f

Yield 67%; m.p. 85–87 °C; ^{1}H NMR (400 MHz, DMSO), 2.51 δ ppm (1H, d, *J* = 13.2 Hz, —CH=CH), 3.48 δ ppm (1H, m, —CH_2-CHOHCH_2), 3.33 δ ppm (4H,dd, *J* = 5.2 Hz, Piprazine), 3.53 δ ppm (1H, d, *J* = 13.1 Hz, —CH=CH), 7.15–7.70 δ ppm (8H, m, Ar—H), 8.13 δ ppm(2H, s, —Ar—CH_2); ^{13}C NMR (100 MHz, DMSO) 20, 73, 122, 126, 128, 130, 138, 140, 185 δ ppm; FTIR (KBr) υ_{max} cm^{-1}: 2912 (C=C), 1645 (C=O), 2221 (C—N, Nitrile), 755 (O-disubstituted Ar).

2.4.7. 4′-4-[3-(4-Chloro-phenyl)-acryloyl]-piperazin-1-ylmethyl-biphenyl-2-carbonitrile 5g

Yield 66%; m.p. 188–189 °C; ^{1}H NMR (400 MHz, DMSO), 2.55 δ ppm (1H, d, *J* = 13.1 Hz, —CH=CH), 3.52 δ ppm (1H, m, —CH_2-CHOHCH_2), 3.38 δ ppm (4H,dd, *J* = 5.3 Hz, Piprazine), 3.58 δ ppm (1H, d, *J* = 13.2 Hz, —CH=CH), 7.22–7.78 δ ppm (8H, m, Ar—H), 8.19 δ ppm(2H, s, —Ar—CH_2); ^{13}C NMR (100 MHz, DMSO) 23, 80, 127, 130, 132, 134, 140, 142, 190 δ ppm; FTIR (KBr) υ_{max} cm^{-1}: 2917 (C=C), 1652 (C=O), 2229 (C—N, Nitrile), 845 (p-disubstituted Ar).

2.4.8. 4′-[4-(3-Naphthalen-1-yl-acryloyl)-piperazin-1-ylmethyl]-biphenyl-2-carbonitrile 5h

Yield 74%; m.p. 127–128 °C; ^{1}H NMR (400 MHz, DMSO), 2.53 δ ppm (1H, d, *J* = 13.3 Hz, —CH=CH), 3.54 δ ppm (1H, m, —CH_2-CHOHCH_2), 3.38 δ ppm (4H,dd, *J* = 5.1 Hz, Piprazine), 3.56 δ ppm (1H, d, *J* = 13.4 Hz, —CH=CH), 7.18–7.72 δ ppm (8H, m, Ar—H), 8.14 δ ppm(2H, s, —Ar—CH_2); ^{13}C NMR (100 MHz, DMSO) 22, 77, 124, 127, 129, 132, 140, 142, 189 δ ppm; FTIR (KBr) υ_{max} cm^{-1}: 2915 (C=C), 1646 (C=O), 2224 (C—N, Nitrile).

2.5. Anti bacterial studies and antifungal studies

All synthesized compounds were screened for their in vitro antibacterial activity (MIC) by broth dilution method against

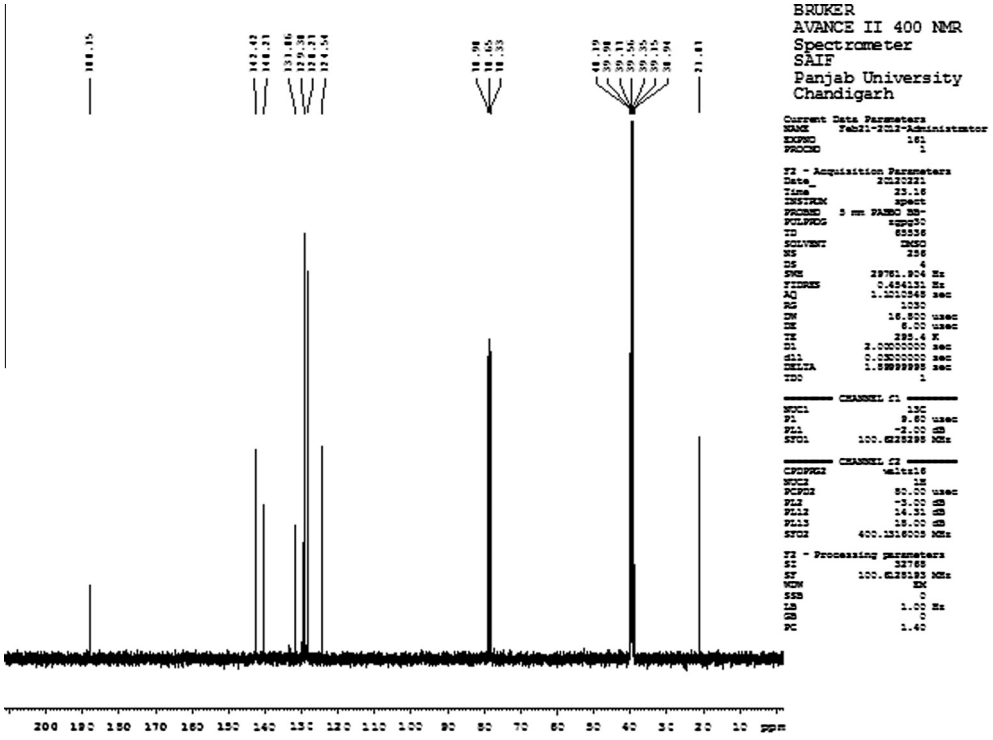

Fig. 3. C^{13} NMR Spectrum of synthesized compound.

Table 3
Antibacterial MIC values of synthesized compounds.

Antibacterial activity table					
Sr. no.	Code no.	*E. coli* MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenus* MTCC 442
Minimum inhibition concentration					
1	5a	100	62.5	200	250
2	5b	200	100	125	100
3	5c	250	250	200	100
4	5d	125	200	125	100
5	5e	200	125	250	250
6	5f	125	100	200	200
7	5g	62.5	100	250	500
8	5h	200	250	500	500

Drug – (μg/ml)	*E. coli* MTCC 443	*P. aeruginosa* MTCC 1688	*S. aureus* MTCC 96	*S. pyogenus* MTCC 442
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	–	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

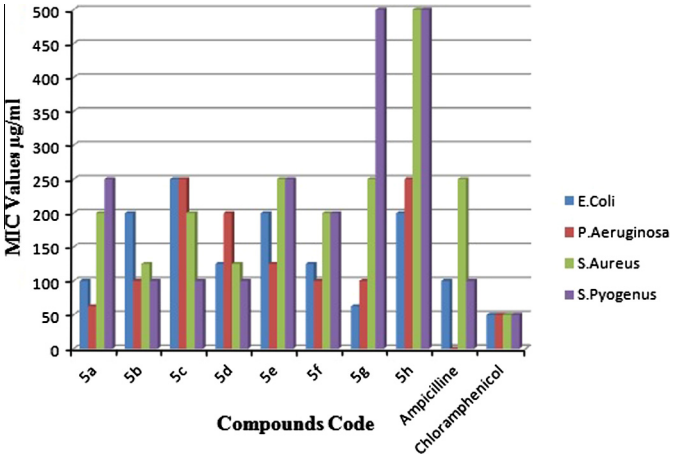

Fig. 4. Bacterial screening.

bacteria E-coli MTCC 443, *P. aeruginosa* MTCC 1688, *S. aureus* MTCC 96, and *S. pyogenus* MTCC 442 using Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamycin, Norfloxacin as a standard drugs. These compounds were also tested for their in vitro antifungal activity (MIC) by the broth dilution method against fungi *C. albicans* MTCC 227 and *A. clavatus* MTCC 1323, using Nystatin and Greseofulvin as a standard drug [22].

3. Results and discussion

Physicochemical parameters of the compounds are presented in Table 1. The melting point of all compounds were determined in open capillary on Veego (Model: VMP-D) electronic apparatus and are uncorrected. All the compounds were colored and stable in air. All synthesized compounds were insoluble in water but soluble in organic solvents like ethylacetae, ethanol, DMF and DMSO.

3.1. FTIR spectra and NMR spectra

FT IR spectra of all synthesized compounds were recorded on Simadzu 8400-S (Japan). The spectrum was run by applying organic sample on KBr cell covering the range of frequencies from 4000 to 400 cm^{-1} with scanning period of 20 s. The spectrophotometer was set at 100% transmittance with pure KBr pellet. Fig. 1 shows typical FT IR spectrum of compound (5d). IR spectrum of the compound 5d showed a characteristic bands between 1640 and 1710 cm^{-1} confirming the presence of C=O group of carbazonyl compounds. Important infrared spectral bands and their tentative

Table 4
Antifungal MIC values of synthesized compounds.

Antifungal activity table			
Sr. no.	Code no.	*C. albicans* MTCC 227	*A. clavatus* MTCC 1323
Minimum inhibition concentration			
1	5a	500	500
2	5b	500	1000
3	5c	>1000	>1000
4	5d	1000	1000
5	5e	>1000	500
6	5f	500	1000
7	5g	500	1000
8	5h	1000	500
Drug (μg/ml)		*C. albicans* MTCC 227	*A. clavatus* MTCC 1323
Nystatin		100	100
Greseofulvin		500	100

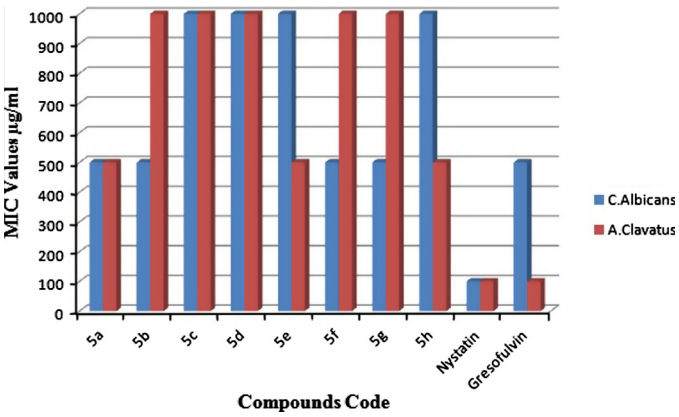

Fig. 5. Fungal screening.

Table 5
Antimalarial MIC values of synthesized compounds.

Sr. no.	Compound code	Mean IC_{50} values (μg/ml)
1	5a	0.097
2	5b	0.56
3	5c	0.098
4	5d	0.45
5	5e	0.42
6	5f	0.073
7	5g	0.043
8	5h	0.083
	Chloro quine	0.02
	Quinine	0.268

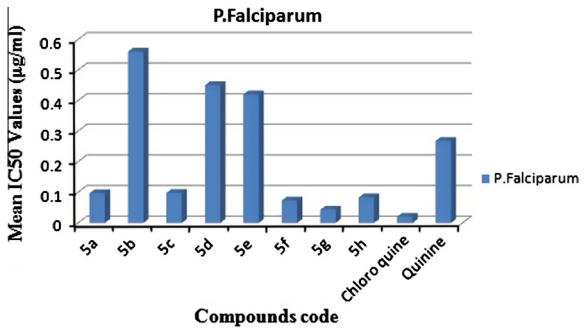

Fig. 6. Malarial screening.

assignments of piprazinyl cyano biphenyl Based compound and its derivatives are summerized in Table 2. ^{1}HNMR and ^{13}C NMR spectra were recorded on Bruker 400 MHz model spectrometer using DMSO and TMS as internal reference (Chemical shifts in δ ppm). ^{1}H NMR spectrum (Fig. 2) of compound 5d revealed signals at 2.37 δ ppm for the Ar—CH_3 group and singlet at 3.37 δ ppm for proton of the Piprazine group. The ^{1}H NMR spectrum of compound 5d revealed signals between 7.18 and 7.73 δ ppm for aromatic protons. The ^{13}C NMR spectrum of compound 5d revealed signals at 124 δ ppm for —CN group and at 128, 129, 131, 140, 142, δ ppm for aromatic carbon (Fig. 3) [23].

3.2. Antibacterial studies

All synthesized compounds were screened for antibacterial study against gram positive and gram negative strain. MIC values of all synthesized compounds were compared with standard drugs viz, Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamycin and Norfloxacin (Table 3). Compound 5 g shows high activity, while compounds 5a, 5d and 5f shows comparable activity with Ampicillin as a standard drug against *E. coli*. Compounds 5a, 5b, 5c, 5d, 5e, 5f and 5g shows comparable activity with Ampicillin as standard drug against *S. aureus*. Compounds 5b, 5c, and 5d shows comparable activity with Ampicillin against *S. pyogenus*. Comparative analysis of antibacterial activity of synthesized compounds and standard drugs shown in Fig. 4.

3.3. Antifungal studies

All synthesized compounds were screened for antifungal study against *C. albicans* and *A. clavatus* strain. MIC values of all synthesized compounds were compared with standard drugs viz, Nystatin and Greseofulvin (Table 4). Compounds 5a, 5b, 5f, and 5g shows comparable activity with Greseofulvin as standard drug against *C. albicans*. Comparative analysis of antifungal activity of synthesized compounds and standard drugs shown in Fig. 5.

3.4. In vitro antimalarial screening

In vitro antimalarial screening was carried out in 96 well microtitre plates according to the microassay protocol of Rieckmann and co-workers with minor modifications. The culture of *P. falciparum* strain was maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage paracetamol of 0.8–1.5% at 3% haematocrit in a total volume of 200 μl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitemia (rings) and uniformly maintained with 50% RBCs (O^{+}). A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 μl volume were added to the test wells so as to obtain final concentrations (at five fold dilutions) ranging between 0.4 μg/ml and 100 μg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37 °C in a candle jar. After 36–40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Chloroquine was used as the reference drug [24–29].

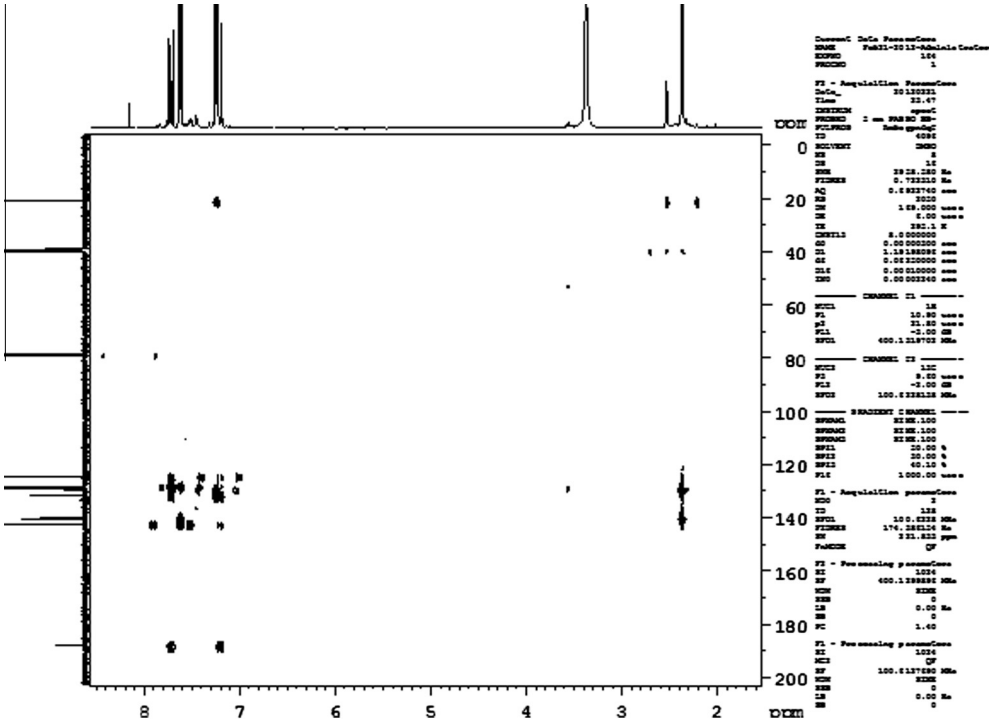

Fig. 7. HMBC Spectrum of synthesized compound.

3.5. Observations of the in vitro antimalarial screening

The mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 h, and percent maturation inhibition with respect to control group. Synthesized compounds were screened against *P. falciparum* strain. Compounds 5a, 5c, 5f, 5g and 5h shows very good IC_{50} values against *P. falciparum* than quinine as a standard drug. The minimum inhibitory concentration (MIC) values of all synthesized compounds shown in the Table 5 and comparative chart in the Fig. 6.

4. SAR study of synthesized compounds

Some of the important milestones of SAR study are outlined here, it is clear that SA accumulation is necessary for the establishment and maintenance of SAR. Cyano biphenyl based piprazinyl compounds is necessary for the broad spectrum antibiotics, towards different bacterial spices like gram +ve and gram −ve. Compounds having —Cl substitution at 2 and 4 position shows good MIC values against gram −ve bacterial spices *E. coli*. Compounds having —NO_2 electron withdrawing group shows good MIC values against gram +ve bacterial species *S. pyogenus*. According to SR of compound having —OCH_3, —Cl groups shows good MIC values against *C. albicans*. SAR study on malarial pathogen *P. falciparum*, compounds having —Cl substitution at 2 and 4 position and —NO_2 substitution at 4-position exhibits very good mean IC_{50} values comparable with quinine (see Fig. 7).

5. Conclusion

The present investigation revealed synthesis of piprazinyl cyano biphenyl based compounds as potential leads for the development of new antibacterial and antimalarial drugs. Compound 5g proved more potent and compound 5a shows comparable activity against *E. coli* as gram −ve bacterial strain as compared to Ampicillin as a standard drug. Compounds 5b, 5c, and 5d shows comparable activity against *S. pyogenus* than Ampicillin as a standard drug. It is also concluded from the results of antifungal activity that compounds 5a, 5b, 5g and 5h shows comparable activity against *C. albicans* as compared to Greseofulvin as a standard drug. Compounds 5a, 5c, 5f, 5g and 5h shows high antimalarial activity against *P. falciparum* as compared to quinine as standard drug. Piprazinyl cyano biphenyl based compounds are more bactericides and fungicides, more over it showed good antimalarial activity. In future piprazinyl cyano biphenyl based derivatives will be used for the further development of the new chemical entity.

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