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Synthesis and bioactivity evaluation of novel bipyenyl thioxo pyrimidines as potent antimicrobial agent

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ABSTRACT

Thioxo Pyrimidine and Biphenyl moieties are well known for their valuable medicinal properties. The present research work describes the synthesis of novel Biphenyl Thioxo Pyrimidine derivatives and evaluation of their medicinal value. The reaction of 4'-Bromomethyl-biphenyl-2-carbonitrile with 4'-Hydroxy acetophenone in presence of Sodium carbonate produced 4'-(4-Acetyl-phenoxymethyl)-biphenyl-2-carbonitrile ***(IN-1).*** *The reaction of* ***IN-1*** *with substituted aromatic aldehyde and Sodium hydroxide afforded various substituted Chalcones; 4'-{4-[3-(2-Chloro-phenyl)-acryloyl]-phenoxymethyl}-biphenyl-2-carbonitrile* ***(IN-2)****. Finally, the reaction of* ***IN-2*** *with Thiourea in presence of Sodium hydroxide produced 4'-{4-[6-(2-Chloro-phenyl)-2-thioxo-2,3-dihydro-pyrimidin-4-yl]-phenoxymethyl}-biphenyl-2-carbonitrile* ***(CB, A-O)****. The chemical structures of synthesized compounds were characterized by* ^{1}*H-NMR,* ^{13}*C-NMR and FT-IR. Synthesized compounds were screened for their antimicrobial activity. The compound CB-G was found to be the most potent antibacterial agent among all synthesized compounds.*

Key Words: Thioxo Pyrimidine, Biphenyl, Chalcone, Antibacterial, Antifungal.

INTRODUCTION

Heterocyclic compounds are abundant in nature and are of great importance to the human being because of their structural subunit exists in many natural products such as vitamins, hormones, antibiotics etc. Among the various heterocyclic, Pyrimidine and Thioxo Pyrimidine are one of the important classes of N-containing heterocyclic compound that have been explored so far for the development of medicinally important compounds. They constitute the future world of therapeutic agent in pharmaceutical chemistry. Pyrimidine and Thioxo Pyrimidine are structural moiety of many natural and synthetic organic therapeutic agents. Synthetic studies of Pyrimidine derivatives have been reported extensively due to their structural diversity and association with wide spectrum of biological value. Pyrimidine is six member ring with nitrogen at 1,3 position.

Extensive research has been reported by number of researcher and screened Pyrimidine derivatives for their potential biological activity. The Pyrimidine compounds are known to posses Anti-inflammatory and Analgesic activity [1-3], Anticancer [4-7], Anti-viral [8-9], Anti-Ulcergenic [10-11], Antitumor [12-14], Anti-HIV [15-17], Anti-Hypertensive [18-19], Anti-Tubercular [20-21], Anti-Malarial [22-23], Anti-Herpes virus [24], Anti Epileptic [25], Anti Parkinsonian [26], Antibacterial [27-29], Cytotoxic [30], Calcium channel blocker [31-32] and Adrenoceptor-selective antagonist [33].

In addition to Pyrimidine moiety, Biphenyl compounds are one of the valuable classes in the organic chemistry which constitutes structural moiety of many pharmaceutical compounds. In past, the use of Biphenyl compounds were limited to chemical and agrochemical industries as an intermediate but, now a days, with advancement in synthetic medicinal chemistry, variety of Biphenyl derivatives were prepared by researchers and evaluated their therapeutic significance. The research data shows that many compounds having Biphenyl moiety are also known to possess Anti-inflammatory [34], Diuretic [35] and Anti-diabetic [36] activity. Some of the Biphenyl containing compounds possesses Antipsychotic and Anxiolytic activity [37]. Some of the Biphenyl hydrazide-hydrazone derivatives are also known to exhibit very good Antimicrobial activity [38-39].

As time advances, the life on the earth faces many challenges to cure the various infections. The increased resistance of microbes to the antimicrobial agent has become the major challenge to the society. The research data states that out of 2 million people who acquired the bacterial infection in US hospital each year, 70% of them involve the strain that are resistant to at least one drug [40]. Even the number of patients with antibiotic resistant infection continues to climb [41]. Despite of extensive research on development of an improved antimicrobial agent, there is urgent need to explore novel, efficient antimicrobial agent.

Being inspired by the requirement of developing efficient antimicrobial agent, the work has been undertaken by for synthesis and evaluation of potential antimicrobial agent. The present work describes the synthesis of novel Biphenyl Thioxo Pyrimidine. Synthesized compounds were screened for antibacterial and antifungal activity.

MATERIALS AND METHODS

Materials:
All key raw materials, reagents and solvents were of commercial grade and pure; used without further purification. All melting points were measured using open capillaries in a liquid paraffin bath and were uncorrected. The completion of reaction was monitored by thin layer chromatography using silica gel-G as absorbent and Toluene: Ethyl acetate was employed as mobile phase. The visualization of TLC was accomplished by UV light and Iodine. IR spectra (KBr pallet) were recorded on FT-IR, Perkin Elmer RX1 spectrophotometer and NMR spectra on BRUKER AVANCE II (400 MHz) using TMS as internal standard (chemical shifts in δ ppm).

Methods:
In the present work, novel Biphenyl Thioxo Pyrimidine derivatives were prepared by following general reaction scheme as shown in figure 1.1. The physical constants of synthesized compounds are mentioned in Table 1.1. Synthesized compounds were screened for antibacterial and antifungal activity. The results of antibacterial and antifungal activity are depicted in Table 1.2 and Table 1.3 respectively.

Procedure for the synthesis of 4'-(4-Acetyl-phenoxymethyl)-biphenyl-2-carbonitrile (IN-1)
A mixture of 4'-Bromomethyl-biphenyl-2-carbonitrile (10 g, 0.037 moles), 4'-Hydroxy acetophenone (5.5 g, 0.040 moles) and Sodium carbonate (7.8 g, 0.074 moles) in Dimethyl formamide (20 ml) were heated at 75-80°C for 4 hours. The progress of the reaction was monitored by Silica gel thin layer chromatography. Toluene: Ethyl acetate (66:33) was used as eluent in TLC chromatography. After completion of reaction, the mass was cooled to 30°C and drawn in 700 ml water. The resultant precipitates were filtered and dried. The crude product was refluxed in 25 ml Methanol, cooled to room temperature and filtered to isolate 4'-(4-Acetyl-phenoxymethyl)-biphenyl-2-carbonitrile as pure, white crystalline powder. The yield of this step was 83% and melting point was 138-140°C.

Procedure for the synthesis of 4'-{4-[3-(2-Chloro-phenyl)-acryloyl]-phenoxymethyl}-biphenyl-2-carbonitrile (IN-2, A-O)
IN-1 (1 g, 3.05 mmoles) and substituted aromatic aldehyde (3.11 mmoles) were dissolved in a binary mixture of Dimethyl formamide and Methanol (1:1). The solution was cooled to 25°C and added 50% Sodium hydroxide (0.48 g, 6 mmoles) over period of 30 minutes under vigorous stirring. Then, the reaction mass was stirred at 25-30°C for 24 hours. The progress of the reaction was monitored by TLC using Toluene: Ethyl acetate (66:33) as eluent. After completion of the reaction, the mass was drawn in water and pH was adjusted to 2 using 16% Hydrochloric acid. Resultant solid was filtered and washed with water till neutral pH is achieved. The crude product was further purified in Methanol [42]. The yield of this step was 80% and melting point was 158-160°C.

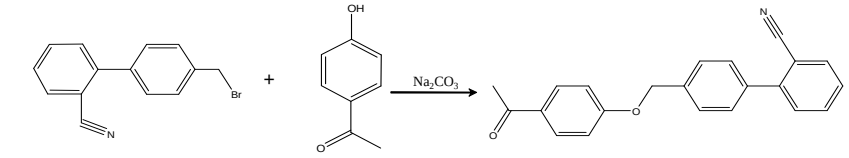
Procedure for the synthesis of 4'-{4-[6-(2-Chloro-phenyl)-2-thioxo-2,3-dihydro-pyrimidine-4-yl]-phenoxymethyl}-biphenyl-2-carbonitrile (CB, A-O)

A mixture of IN-2 (A-O) (2 g, 6.1 mmoles), Thiourea (6 g, 79.94 mmoles), 25% Sodium hydroxide (11 g, 68.75 mmoles) in 90% Methanol were heated at 65-70°C for 48 hours. The progress of the reaction was monitored by TLC using Toluene: Ethyl acetate as eluent. After completion of the reaction, the mass was cooled to 30°C and filtered to remove insoluble. The filtrate was drawn in 500 ml water and pH of the mass was adjusted to 7.0-7.5 using 16% Hydrochloric acid. The precipitated solids were isolated by filtration and dried. The crude product was further purified in Methanol and then in Ethyl acetate. The yield of this step was 68% and melting point was 188-190°C.

REPRESENTATIVE SPECTRAL DATA

4'-{4-[6-(2-Chloro-phenyl)-2-thioxo-2,3-dihydro-pyrimidine-4-yl]-phenoxymethyl}-biphenyl-2-carbonitrile (CB-A)

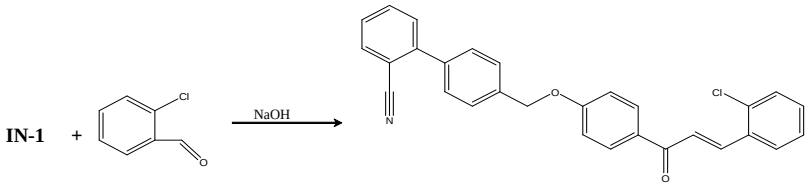
^{1}H NMR (DMSO) δ ppm: 4.7 (2H, s, -O-C<u>H</u>$_2$-Ph), 5.08 (1H, s, =C<u>H</u>, heterocyclic), 5.2 (1H, s, -N<u>H</u>, heterocyclic), 7.01-8.21 (16H, m, Ar-H). ^{13}C NMR (DMSO) δ ppm: 78.6 (-<u>C</u>H$_2$-O), 97.26 (-<u>C</u>H=C-, heterocyclic), 158.75 (=<u>C</u>-NH-, heterocyclic ring), 110-161 (Aromatic -C), 175.99 (><u>C</u>=N-, heterocyclic), 195.91 (-<u>C</u>=S, heterocyclic). FT-IR, (KBr, cm^{-1}): 760 (o-substituted Benzene), 824 (p-Substituted Benzene), 1036 (Ar-Cl), 1184 (-C-O-, ether), 1665 (->C=S), 2224 (C≡N), 3403 (-NH, heterocyclic).



4'-Bromomethyl-biphenyl-2-carbonitrile

4'-(4-Acetyl-phenoxymethyl)-biphenyl-2-carbonitrile

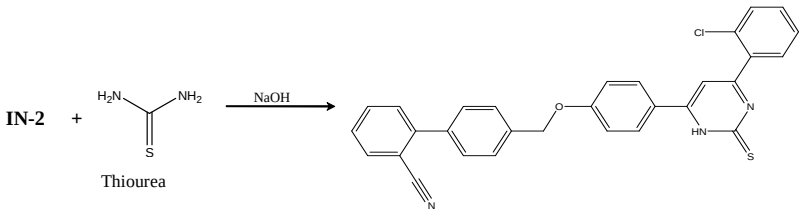
IN-1



Benzaldehyde

IN-2

4'-{4-[3-(2-Chloro-phenyl)-acryloyl]-phenoxymethyl}-biphenyl-2-carbonitrile



Thiourea

4'-{4-[6-(2-Chloro-phenyl)-2-thioxo-2,3-dihydro-pyrimidin-4-yl]-phenoxymethyl}-biphenyl-2-carbonitrile

CB-A

Figure: 1.1: General Synthesis scheme

Table 1.1: Physical constants of synthesized compounds

Sr. No	Compound Name	R	R^1	R^2	Molecular Formula	Mol. Weight	Melting Point [°C]	Rf Value
1	CB-A	Cl	H	H	$C_{30}H_{20}ClN_3OS$	506.02	188-190	0.14*
2	CB-B	H	H	Cl	$C_{30}H_{20}ClN_3OS$	506.02	157-159	0.12*
3	CB-C	H	Cl	H	$C_{30}H_{20}ClN_3OS$	506.02	178-180	0.55**
4	CB-D	H	H	CH_3	$C_{31}H_{23}N_3OS$	485.16	133-135	0.16*
5	CB-E	CH_3	H	H	$C_{31}H_{23}N_3OS$	485.16	158-160	0.61**
6	CB-F	H	H	H	$C_{30}H_{21}N_3OS$	471.14	124-128	0.36*
7	CB-G	Br	H	H	$C_{30}H_{20}BrN_3OS$	549.05	118-121	0.19*
8	CB-H	H	Br	H	$C_{30}H_{20}BrN_3OS$	549.05	103-105	0.14*
9	CB-I	H	H	Br	$C_{30}H_{20}BrN_3OS$	549.05	98-101	0.54**
10	CB-J	H	H	OCH_3	$C_{31}H_{23}N_3O_2S$	501.15	143-145	0.60**
11	CB-K	OCH_3	H	H	$C_{31}H_{23}N_3O_2S$	501.15	168-170	0.58**
12	CB-L	H	H	$N(CH_3)_2$	$C_{32}H_{26}N_4OS$	514.18	135-138	0.61**
13	CB-M	H	OCH_3	OCH_3	$C_{32}H_{25}N_3O_3S$	531.16	145-147	0.53**
14	CB-N	H	NO_2	H	$C_{30}H_{20}N_4O_3S$	516.13	185-188	0.58**
15	CB-O	H	H	NO_2	$C_{30}H_{20}N_4O_3S$	516.13	168-171	0.60**

**Toluene: Ethyl acetate : : 95 : 5, **Toluene: Ethyl acetate : : 66 : 33*

BIOLOGICAL EVALUATION

Among all synthesized compounds, selected compounds were evaluated for their in vitro antibacterial and antifungal activity using representative strains of Gram-negative bacteria (Escherichia Coli, Pseudomonas Aeruginosa) and Gram-positive bacteria (Staphylococcus Aureus, Streptococcus Pyogenus). For antifungal activity, Candida Albicans, Aspergillus Niger and Aspergillus Clavatus were used as representative stains. The Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin were used as standard antibacterial drugs for the comparison. While, Nystatin and Greseofulvin were used as standard antifungal drugs. The Agar diffusion and broth dilution test method were followed for evaluation of antimicrobial activity. The test compounds were dissolved in Dimethyl Sulfoxide (DMSO) and Muller Hinton Broth was used as nutrient medium to grow and dilute the drug suspension for the test bacteria. Serial dilutions were prepared for primary and secondary screening for the test compounds. The test compound tubes were incubated for 24 hours at 37ºC and turbidity produced in each tube was recorded by UV/Visible spectrophotometer. The turbidity produced by the Broth (without inoculums) was considered as 100% transparency. The minimum inhibitory concentration (MIC) was noted as the minimum concentration of the test substance, which completely inhibits the growth of the microorganism i.e. 100% transparency.

RESULTS AND DISCUSSION

In the present study, fifteen novel Biphenyl Thioxo Pyrimidine compounds were synthesized in reasonably good yield. The presence of characteristic peaks at 1665 and 3403 cm^{-1} in FT-IR confirmed the presence of Thioxo Pyrimidine ring. The presence of ether link was confirmed by characteristic peak at 1184 cm^{-1} in FT-IR spectra. The characteristic peak observed at 2224 cm^{-1} confirmed the presence of nitrile group. Further, the structure of compound was confirmed by ^{1}H-NMR and ^{13}C-NMR. Selected synthesized compounds were evaluated for their antibacterial and antifungal activity. The test results of antimicrobial activity are mentioned in Table 1.2, Table 1.3 and in Graph 1.1, Graph 1.2.

(1) Antibacterial evaluation: Compounds **CB-G, CB-L, CB-M** are found equipotent to Ampicillin (MIC=100 μg/mL) against E.Coli and P.Aeruginosa (gram -ve). Compounds **CB-G, CB-L, CB-M and CB-N** possessed very good antibacterial activity against S.Aureus (gram +ve) and found more efficient than Ampicillin (MIC=250 μg/mL). Compounds **CB-A, CB-F, CB-H** and **CB-J** are found to Ampicillin against S.Aureus. Compounds **CB-G** is found equipotent to Ampicillin (MIC=100 μg/mL) against S.Pyogenus (gram +ve). Overall, compound CB-G possesses moderate to good antibacterial activity against gram positive and gram negative bacteria.

(2) Antifungal evaluation: Compounds **CB-A, CB-H, CB-J, C-L and CB-N** are found equipotent to Greseofulvin (MIC=500 μg/mL) against C.Albicans. While, all other synthesized compounds are less potent than standard drugs.

Table 1.2: Antibacterial Activity, Minimum Inhibition Concentration. (MIC[a])

Compound Name	E. Coli (MIC)a	P. Aeruginosa (MIC)a	S.Aureus (MIC)a	S.Pyogenus (MIC)a
	MTCC 443 Gram-Negative	MTCC 441 Gram-Negative	MTCC 96 Gram Positive	MTCC 442 Gram-Positive
CB-A	125	125	**250**	200
CB-B	200	250	500	500
CB-D	125	125	500	500
CB-F	200	250	**250**	250
CB-G	**100**	250	**62.5**	**100**
CB-H	500	500	**250**	200
CB-J	500	250	**250**	200
CB-L	125	**100**	**200**	200
CB-M	200	**100**	**100**	125
CB-N	125	250	**200**	200
Standard drugs				
Ampicillin	**100**	**100**	**250**	**100**
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

(MIC)[a]: Minimum Inhibitory concentration in µg/ml

Graph 1.1: Antibacterial Activity, Minimum Inhibition Concentration (Graphical form)

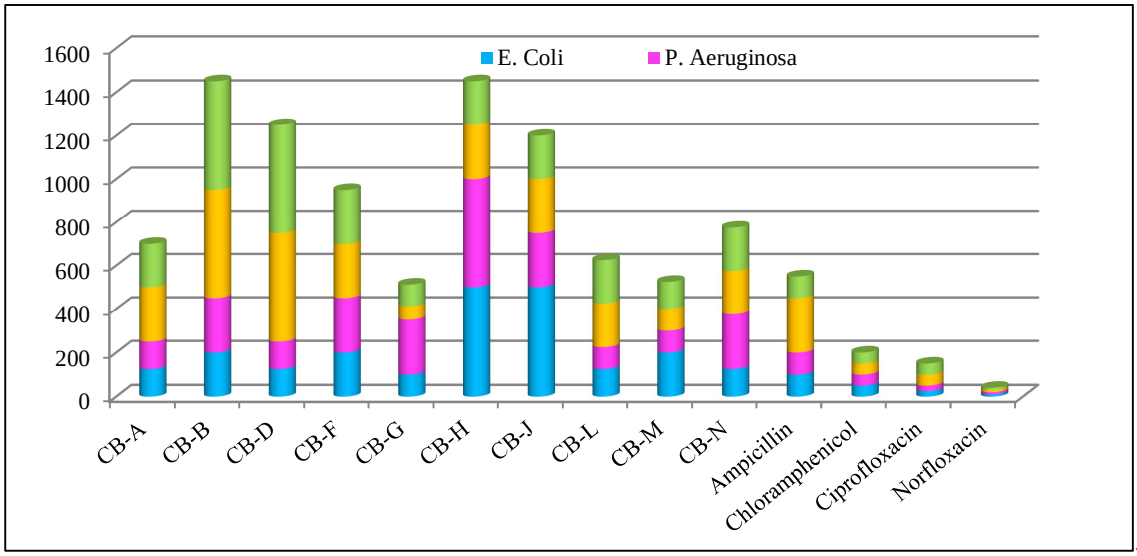
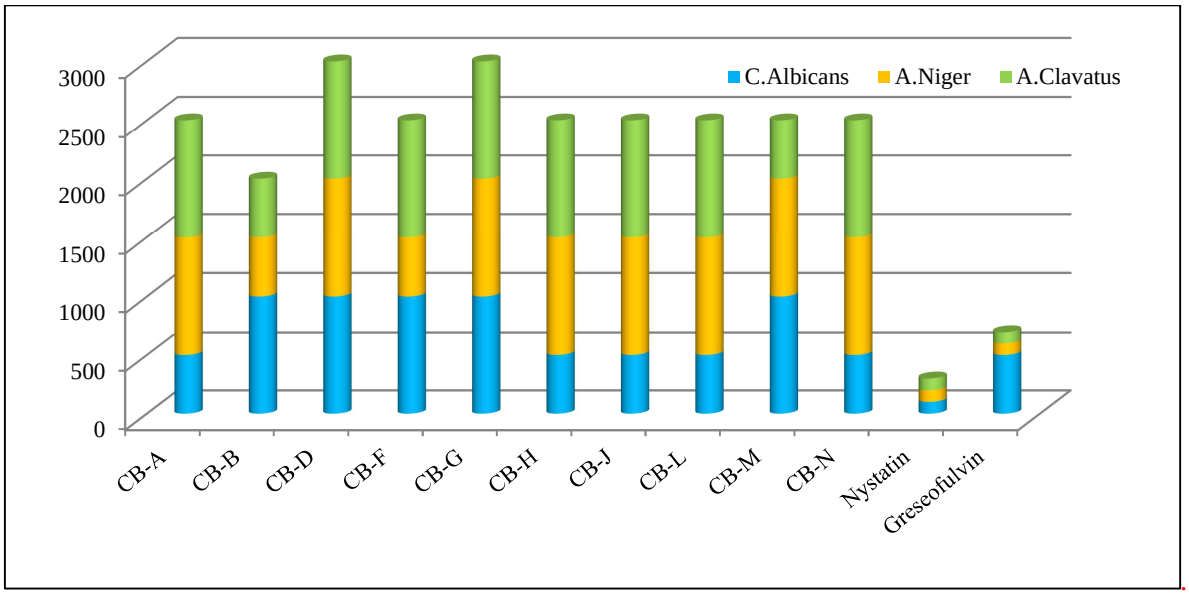


Table 1.3: Antifungal activity, Minimum Fungicidal Concentration

Compound Name	C.Albicans (MIC)b	A.Niger (MIC)b	A.Clavatus (MIC)b
	MTCC 227	MTCC 282	MTCC 1323
CB-A	**500**	1000	>1000
CB-B	1000	500	500
CB-D	>1000	>1000	>1000
CB-F	1000	500	1000
CB-G	>1000	1000	1000
CB-H	**500**	1000	>1000
CB-J	**500**	>1000	1000
CB-L	**500**	>1000	1000
CB-M	1000	>1000	500
CB-N	**500**	>1000	>1000
Standard drugs			
Nystatin	100	100	100
Greseofulvin	**500**	100	100

(MIC)[b]: Minimum Inhibitory concentration in µg/ml

Graph 1.2: Antifungal activity, Minimum Fungicidal Concentration (Graphical Form)



CONCLUSION

The novel Biphenyl Thioxo Pyrimidine compounds can be synthesized in reasonably good yield using commercial grade raw materials. The structures of synthesized compounds were confirmed by FT-IR, ^{1}H-NMR and ^{13}C-NMR. The synthesized compounds were evaluated for biological property. The compound CB-G was found as the most potent antibacterial agent among all synthesized compounds.

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