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SYNTHESIS AND ANTIMICROBIAL STUDIES OF BIPHENYL-4-CARBOXYLIC ACID 2-(ARYL)-4-OXO-THIAZOLIDIN-3-YL – AMIDE

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ABSTRACT : Some derivatives of biphenyl-4-carboxylic acid 2-(aryl)-4-oxo-thiazolidin-3-yl –amide were synthesized and studied for their antimicrobial activity. These compounds were prepared from biphenyl-4-carboxylic-acid hydrazides. Biphenyl-4-carboxylic-acid hydrazides 1 on refluxing with aryl aldehydes in the presence of catalytic amount of glacial acetic acid furnish the biphenyl-4-carboxylic acid hydrazone. The aryl hydrazones II on reaction with thioglycolic acid in the presence of anhydrous zinc chloride yielded the biphenyl-4-carboxylic acid-2-(aryl)-4-oxo-thiazolidin-3-yl-amides III. These compounds were characterised by CHN analyses, IR, and ^{1}H NMR spectral data. All the compounds were evaluated for their in vitro antimicrobial activity against two Gram negative strains (Escherichia coli and Pseudomonas aeruginosa) and two Gram positive strains (Bacillus subtilis and Staphylococcus aureus) and also against fungi Candida albicans and Aspergillus Niger. All compounds show promising results.
Keywords: Synthesis, Antimicrobial activity, Minimum inhibitory concentration.

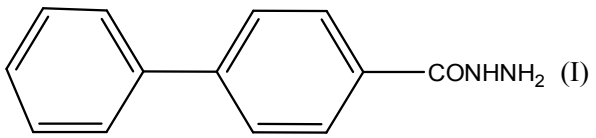
INTRODUCTION

The extensive use of antibiotics has led to the appearance of multi-drug resistant microbial pathogens.[1, 2] This highlights the incessant need for the development of new classes of antimicrobial agents and alteration of known drugs in such way that would allow them to retain their physiological action, but reducing their resistance to the pathogen. The design of novel chemotherapeutic agents is particularly beneficial due to their dissimilar mode of action which can avoid cross resistance to known drugs. 4-thiazolidinones have received considerable attention due to their wide range of biological activities. 4-Thiazolidinone derivatives are known to possess antimicrobial [3-7], analgesic [8], anti-inflammatory [9, 10], anti-HIV activity [11], cytotoxic activity [12], anticonvulsant [13], antiarrhythmic [14] etc. 4-thiazolidinone has been found as novel inhibitor of bacterial enzyme MurB, a key enzyme responsible for the synthesis of peptidoglycon [15]. These reports prompted us to synthesize the novel derivatives of 4-thiazolidinone incorporating known bioactive heterocyclic nuclei such as thiazolidinone.

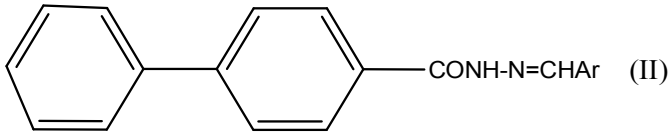
EXPERIMENTAL WORK

The purity of the synthesized compounds were ascertained by thin layer chromatography on silica gel G in various solvent systems using iodine vapors as detecting agent. Melting points were determined by Toshniwal Melting Point in open capillary tubes and are uncorrected. Elemental analyses were done using Carlo Erba 1106 CHN Analyzer. Infra-red spectra were recorded on Perkin Elmer Spectrum RXI FTIR spectrophotomer in KBr phase. Proton NMR spectra were recorded on Bruker Avance II 400 NMR Ultra Shield Spectrometer using tetramethyl silane as internal standard.

4-Thiazolidinone may be conveniently prepared by the reaction of thioglycolic acid with substituted acid hydrazide In the presence of few drops of glacial acetic acid.



Biphenyl-4-carboxylic acid hydrazide



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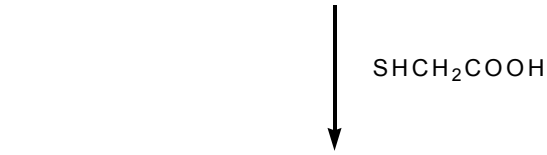

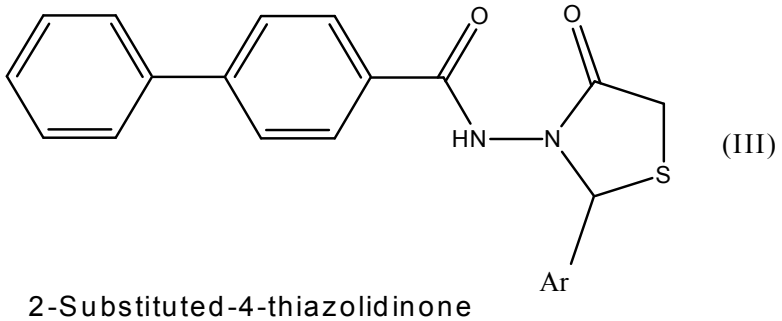

2-Substituted-4-thiazolidinone

2a	C_6H_5-	2h	3-ClC_6H_4
2b	3-$NO_2C_6H_4$	2i	4-$OCH_3C_6H_4$
2c	3-OHC_6H_4	2j	3-$OCH_3C_6H_4$
2d	4-OHC_6H_4	2k	3-BrC_6H_4
2e	4-FC_6H_4	2l	2, 3-di-$OCH_3C_6H_3$
2f	2-ClC_6H_4	2m	2, 4-di-ClC_6H_3
2g	4-ClC_6H_4	2n	2, 5-di-ClC_6H_3

Scheme 1

Synthesis of Biphenyl-4-Carboxylic acid 2-(Aryl)-4-oxo-thiazolidin-3-yl –amide.

1. Synthesis of Biphenyl-4-Carboxylic acid hydrazide hydrazones:

A mixture of (0.025 M) biphenyl-4-carboxylic acid hydrazide and required aromatic aldehydes (0.025 M) was refluxed in methanol (50 ml) in the presence of a catalytic amount of glacial acetic acid for about 2 hrs. The mixture was cooled; the solid was separated by filtration and recrystallized from methanol to give the corresponding hydrazide hydrazones. (Yield is 86.79%, 36.8 gm, R_f is 0.69, m. p. is 182-183 ^oC.)

2. Synthesis of 2-substituted-4-thiazolidinone:

A mixture of (0.015M) biphenyl-4-carboxylic acid hydrazide hydrazone and required amount of thioglycolic acid (0.015 M) in DMF (50 ml), containing a pinch of anhydrous $ZnCl_2$ was refluxed for about 6 hrs. The reaction mixture was cooled and poured on to crushed ice. The solid thus obtained was filtered, washed with water and the product was re-crystallized from rectified spirit.

1. **Biphenyl-4-Carboxylic acid (4-oxo-2-phenyl-thiazolidin-3-yl)-amide.**

Yield: 79%; m.p.: 216 – 218 ºC; IR (cm^{-1}, KBr): 3252 (N–H str amide1), 3051 (C–H str aromatic), 1656 (C=O str amide1), 1609-1482 (C=C str aromatic), 1447-1434 (C–N str), 1581-1529; ^{1}HNMR, ppm (DMSO): 8.02 (s, 1H, -NH), 7.78-7.47 (m 9H, Ar, H), 7.42 -7.42 (m, 4H, Ar H), 6.02(s, 1H, -NCHS), 3.34 (s, 1H, -CHS). Anal.: Calcd. For $C_{22}H_{18}N_2O_2S$: C, 70.57; H, 4.85; N, 7.48.Found C, 70.62; H, 4.82; N, 7.49.

2. Biphenyl-4-Carboxylic acid [2-(3-nitro-phenyl)-4-oxo-thiazolidin-3-yl]-amide.

Yield: 80%; m.p.: 195-197 ºC; IR (cm^{-1}, KBr): 3229 (N–H str amide1), 3028 (C–H str aromatic), 1650 (C=O str amide1), 1606-1484 C=C str aromatic), 1446-1402 (C–N str), 1549 (N–O str, NO_2);^{1}HNMR, ppm (DMSO): 8.04-7.42 (m, 9H, Ar H), 8.02 (s, 1H, -NH), 7.38-6.94 (m, 4H, Ar', H), 6.80 (s, 1H, -NCHS), 3.82(s, 1H, -CHS). Anal.: Calcd. For $C_{22}H_{17}N_3O_4S$: C, 63.00; H, 4.09; N, 10.02. Found C, 63.06; H, 4.03; N, 10.06.

3. Biphenyl-4-Carboxylic acid [2-(4-methoxy-phenyl)-4-oxo-thiazolidin-3-yl]-amide

Yield: 78%; m.p.: 226-228 ºC; IR (cm^{-1}, KBr): 3206 (N–H str amide1), 3038 (C–H str aromatic), 1652 (C=O str amide1), 1606-1474 (C=C str aromatic), 1443-1404 (C–N str), 1279-1218(C–O–C str);^{1}HNMR, ppm (DMSO): 8.05-7.44 (m, 9H, Ar H), 8.02 (s, 1H, -NH), 7.39-6.82 (m, 4H, Ar', H), 6.90 (s, 1H, -NCHS), 3.83(s, 1H, -CHS), 4.08 (s, 1H, -OCH). Anal.: Calcd. For $C_{23}H_{20}N_2O_3S$: C, 68.30; H, 4.98; N, 6.93. Found C, 68.26; H, 4.99; N, 6.89.

4. Biphenyl-4-Carboxylic acid [2-(3-bromo-phenyl)-4-oxo-thiazolidin-3-yl]-amide

Yield: 74%; m.p.: 214-216 ºC; IR (cm^{-1}, KBr): 3207 (N–H str amide1), 3032 (C–H str aromatic), 1647 (C=O str amide1), 1606-1483 (C=C str aromatic), 1442-1402 (C–N str), 554-506 (C–Br);^{1}HNMR, ppm (DMSO): 8.48-7.40 (m, 9H, Ar H), 8.04 (s, 1H, -NH), 7.66-7.28 (m, 4H, Ar' H), 6.02 (s, 1H, -NCHS), 3.81(s, 1H, -CHS). Anal.: Calcd. For $C_{22}H_{17}BrN_2O_2S$: C, 58.28; H, 3.78; N, 6.18. Found C, 58.29; H, 3.74; N, 6.21.

5. Biphenyl-4-Carboxylic acid [2-(4-chloro-phenyl)-4-oxo-thiazolidin-3-yl]-amide

Yield: 91%; m.p.: 222-224 ºC; IR (cm^{-1}, KBr): 3251 (N–H str amide1), 3048 (C–H str aromatic), 1657 (C=O str amide1), 1606-1482 (C=C str aromatic), 1404 (C–N str), 779-604(C–Cl);^{1}HNMR, ppm (DMSO): 8.43-7.44 (m, 9H, Ar H), 8.00 (s, 1H, -NH), 7.52-7.26 (m 4H, Ar' H), 6.07 (s, 1H, -NCHS), 3.56(s, 1H, -CHS). Anal.: Calcd. For $C_{22}H_{17}ClN_2O_2S$: C, 64.62; H, 4.19; N, 6.85. Found C, 64.59; H, 4.22; N, 6.83.

Antimicrobial evaluation

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against Gram positive *S. aureus* (MTCC 121), *B. subtilis* (MTCC 96), and Gram negative *E. coli* (MTCC 40), *P. aeruginosa* (MTCC 2453) and also against fungi *C. albicans* (MTCC 8184) and A. *Niger* (MTCC 8189). Antimicrobial activity was assessed by serial two fold dilution technique. Ciprofloxacin was used as a standard drug for antibacterial activity and clotrimazole was used as a standard drug for antifungal activity. All the compounds were dissolved in dimethyl sulfoxide to give a concentration of 10 µg ml^{-1}. Double strength nutrient broth was used as a growth media.

The stock solution was serially diluted to give concentrations of 2.5-0.156 µg ml^{-1} in nutrient broth. The inoculum size was approximately 10^6 colony forming units (CFU/ml). The tubes were incubated at 37 ± 1°C for 24 h (bacteria) and 25°C for 7 d (*A. Niger*). After that, the inoculated culture tubes were macroscopically examined for turbidity. The culture tube showing turbidity (lower concentration) and the culture tube showing no turbidity (higher concentration) gave the minimum inhibitory concentration (MIC) for the compound. The MIC for antibacterial is given in Table 1 and MIC for antifungal is given in Table 2.

RESULTS AND DISCUSSION

Chemistry

The syntheses of Biphenyl-4-Carboxylic acid 2-(Aryl)-4-oxo-thiazolidin-3-yl –amide were achieved following the steps outlined in Scheme 1. Biphenyl-4-carboxylic acid hydrazide **I** was converted to the corresponding aryl hydrazones **II** using aryl aldehydes in the presence of catalytic amount of glacial acetic acid. The aryl hydrazones **II** on reaction with thioglycolic acid in the presence of anhydrous zinc chloride yielded the biphenyl-4-carboxylic acid-2-(aryl)-4-oxo-thiazolidin-3-yl-amides **III** in good yields.

Infrared spectra of each compound showed NH amide stretching vibrations in the range of 3252-3206 cm^{-1}. The C=O stretching vibrations for amide group was

absorbed in the range of 1657-1647 cm^{-1}. The C-N stretching vibrations of 4-thiazolidinone was absorbed in the range of 1447-1402 cm^{-1}. In case of ^1H NMR, the value of NH amide of 4-thiazolidinone of each compound was found in the range of 8.04-8.02 δ (ppm) and appeared as singlet (s). The value of biphenyl-4-carboxylic acid was found in the range of 8.48-7.40 δ (ppm) and appeared as multiplet (m). The proton of –CHS of 4-thiazolidinone was found in the range of 3.83-3.34 δ (ppm) and appeared as singlet (s). The results of elemental analyses were found in good agreement with the calculated values.

The elemental analysis, IR and ^{1}HNMR spectral data of synthesized compounds were found in agreement with the assigned molecular structure.

The newly obtained derivatives were evaluated for their in vitro antibacterial activity against Gram positive Bacillus subtilis, Staphylococcus aureus, Gram negative Escherichia coli and antifungal activity against Candida albicans and Aspergillus Niger.

All the compounds showed appreciable in vitro antimicrobial activity against the Microorganisms under study.

Minimum Inhibitory Concentration (MIC)

The reference standard ciprofloxacin inhibited Gram negative bacteria E. *coli* and P. *aeruginosa* at a MIC of 0.01 μg ml^{-1} and 0.25 μg ml^{-1}, respectively whereas against Gram positive bacteria S. aureus and B. *subtilis* MIC was found to be 0.15 μg ml^{-1} and 0.12 μg ml^{-1}, respectively and the reference standard clotrimazole inhibit fungi *C. albicans* and A. *Niger* at a MIC of 0.10 μg ml^{-1} and 0.30 μg ml^{-1}, respectively.

All the synthesized compounds 2a-2n showed significant antimicrobial activity, against bacterial strain, E. *coli.* (MIC 2.50-0.31 μg ml^{-1}), P. *aeruginosa* (MIC 2.50-0.62 μg ml^{-1}), S. *aureus* (MIC 1.25-0.31 μg ml-1) and B. *subtilis* (MIC 2.50-0.31 μg ml-1) as compared to the standard drug ciprofloxacin (Table 1) and against fungal strain, *C. albicans* (MIC 1.25-0.31) and A. *Niger* (2.50-0.62) as compared to the standard drug clotrimazole Table 2. Compounds containing 3-NO_2, 2-Cl, and 4-Cl moiety (2b, 2f and 2g) were found to be most active. The results of the MIC for the standard drugs, ciprofloxacin and clotrimazole against the bacterial and fungal strains used were found to be within the range as reported in literature [16-18].

In conclusion, we have described a straightforward synthesis of new biphenyl-4-carboxylic acid 2-(aryl)-4-oxo-thiazolidin-3-yl –amide and studied their in vitro antimicrobial activity. Compounds 2b, 2f and 2g exhibited significant activity against all the bacterial and fungal strains used in this study.

Table 1. *In Vitro* Antibacterial Activity of the Title Compounds (2a-n).

Compound	Minimum inhibitory concentration (μg ml^{-1})			
	E. coli **(MTCC 40)**	***P. aeruginosa*** **(MTCC 2453)**	***S. aureus*** **(MTCC 121)**	***B. subtilis*** **(MTCC 96)**
2a	2.50	1.25	1.25	2.50
2b	0.31	1.25	0.62	0.31
2c	2.50	2.50	1.25	1.25
2d	1.25	0.62	1.25	0.62
2e	1.25	0.62	0.62	2.50
2f	2.50	1.25	0.31	0.31
2g	1.25	0.31	1.25	0.31
2h	0.62	1.25	1.25	1.25
2i	1.25	0.62	1.25	1.25
2j	1.25	0.62	1.25	1.25
2k	0.62	1.25	2.50	1.25
2l	2.50	1.25	1.25	0.62
2m	1.25	1.25	2.50	1.25
2n	2.50	1.25	1.25	1.25
Ciprofloxacin (standard drug)	0.01	0.25	0.15	0.12

Table 2. *In Vitro* Antifungal Activity of the Title Compounds (2a-n).

Compound	Minimum inhibitory concentration (µg ml^{-1})	
	C. albicans **(MTCC 8184)**	**A. *Niger*** **(MTCC 8189)**
2a	1.25	2.50
2b	0.31	1.25
2c	0.62	2.50
2d	1.25	1.25
2e	1.25	2.50
2f	0.31	1.25
2g	0.62	0.62
2h	1.25	1.25
2i	1.25	1.25
2j	2.50	1.25
2k	1.25	1.25
2l	0.62	1.25
2m	1.25	0.62
2n	2.50	1.25
Clotrimazole (standard drug)	0.10	0.30

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