Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2011, 3(3):323-329
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis and biological evaluation of dibenzo[*b,f*]azepine-5-carboxylic acid[1-(substituted-phenyl)-ethylidene]-hydrazides

Gopal Krishna Rao[a*], Ranjit Kaur [a], PN Sanjay Pai [b]

[a]*Department of Pharmaceutical Chemistry, Al-Ameen College of Pharmacy, Bangalore*
[b]*Department of Quality Assurance, Al-Ameen College of Pharmacy, Bangalore*

ABSTRACT

A series of some new Schiff bases of Dibenzo[b,f]azepine-5-carboxylic acid[1-(substituted-phenyl)-ethylidene]-hydrazide ***(4a-j)*** *were synthesized using various substituted aromatic ketones in absolute ethanol with few drops of glacial acetic acid as a catalyst. The structures were confirmed by their IR, and* ^{1}*H NMR spectral and CHN analytical data. All the derivatives have been evaluated for their anti-bacterial activity by cup plate method and antitubercular activity by microbroth dilution method. Some of the derivatives have shown moderate to good biological activity.*

Key words: Dibenzo[b,f]azepine-5-carboxylic acid[1-(substituted-phenyl)-ethylidene]-hydrazide, Schiff bases, hydrazones, antibacterial, antitubercular activity.

INTRODUCTION

Schiff bases are condensation products of primary amines and aromatic aldehydes. Schiff bases and azo compounds are important structures in the medicinal and pharmaceutical fields and it has been suggested that the azomethine linkage might be responsible for the biological activities displayed by Schiff bases [1].

In recent years, there has been an increasing interest in the design and development of Schiff base derivatives. A large number of heterocyclic Schiff bases have been reported to have bactericidal, fungicidal, antipyretic, antitumor, antitubercular, anticancer and sterease inhibitory activities. Also Schiff's bases and farmazones have shown antiviral, antimicrobial and anti-inflammatory activities [2-7]. Further-more hydrazones are associated with broad spectrum biological activities. As a result of these useful properties, a large number of Schiff bases have been developed [8].

These findings prompted us to synthesize some new Schiff bases possessing antimicrobial and antitubercular activities. Thus the present work is focused on the synthesis of substituted phenyl carbonyl hydrazones of dibenzazepine (**4a-j)**.

The synthesis involves the acid-catalysed condensation of 5*H*-dibenzo (b,f)azepine-5*H*-acid hydrazide **(2)** with substituted aromatic ketones in ethyl alcohol containing catalytic amount of glacial acetic acid.

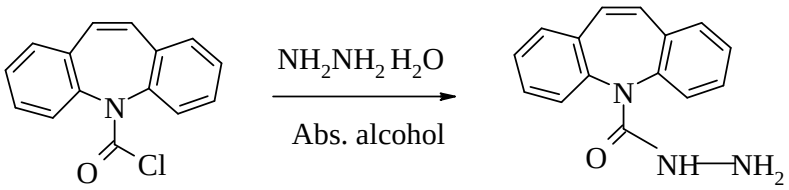
The intermediate 5*H*-dibenzo (b,f) azepine-5*H*- acid hydrazide **(2)** was obtained by condensing 5*H*-dibenzo (b,f) azepine-5*H*- carbonyl chloride and hydrazine hydrate in ethanol.

Formation of the products was checked by difference in m.p and R_f values and confirmed by spectral studies. The IR spectra showed the absence of NH_2 doublet, presence of C=O of amide at 1698 -1676 $cm^{-1}$and formation of azomethine linkage (C=N str), at 1589-1605 cm^{-1}. Formation of the compounds 4a, 4c, 4f and 4h has been authenticated by their ^{1}HNMR spectra showing multiplet for aromatic protons at δ value of 8.95-6.61 and singlet for NH at 9.6-8.7 respectively. The compounds were further confirmed by satisfactory CHN analysis.

MATERIALS AND METHODS

Melting points of the synthesized compounds were determined using open capillary tubes and are uncorrected. The IR spectra of the synthesized compounds were recorded using KBr pellets in range of 4000-400 cm^{-1} on a Fourier Transform IR Spectrometer (Shimadzu 8700) and the frequencies are recorded in wave numbers. ^{1}H-NMR (400 MHz) spectra were recorded in $CDCl_3$-d_6 in Amx-400 liquid state PMR spectrometer (Astra Zeneca, Bangalore). Chemical shifts (δ) are reported in parts per million downfield from internal reference tetramethylsilane (TMS). The ^{13}C-NMR spectra were obtained at 75.4 or 100.59 MHz in $CDCl_3$. The physical constants of the products are reported in **Table – 1**.

Step 1: 5*H*-dibenzo (b, f) azepine-5*H*-acid hydrazide (2)

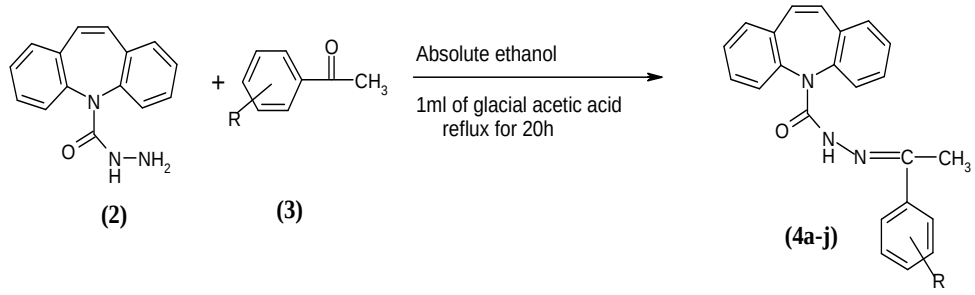


(1) **(2)**

5*H*-Dirbenzo (b,f) azepine-5-acid hydrazide (2).

A mixture of 5*H*-dibenzo(b,f) azepine -5-carbonyl chloride (1) (0.01 mole) and hydrazine hydrate (0.01 mole, 80%) in absolute ethanol was stirred for 1hr and then refluxed for 30min on a water bath. The contents were cooled and product obtained was filtered, washed with cold ethanol, dried and purified by recrystallization from methanol to give 5*H*-Dirbenzo (b,f) azepine-5-acid hydrazide. Yield 75%, m.p 178^{0}C. TLC (methanol: toluene, 2:8, R_f (0.20)

Step 2: Dibenzo[b,f]azepine-5-carboxylic acid[1-(substituted-phenyl)-ethylidene]-hydrazide (4a-j)
PROCEDURE



IR (KBr) cm^{-1} ν :3470, 3352 (NH_2 str), 3019 (Ar-H str), 1647 (C=O str), 1601, 1566 and 1452 (Ar-C=C def). ^{1}HNMR ($CDCl_3$).δ (ppm): 7.35 (m, 8H, Ar-H), 6.87(s, 2H,CH=CH), 5.85(bs, 1H, -NH), 3.47 (bs, 2H, -NH_2).

PROCEDURE

Dibenzo[b,f]azepine-5-carboxylic acid[1-(substituted-phenyl)-ethylidene]-hydrazide (4a-j).

A mixture of 5*H*-dibenzo(b,f) azepine-5*H*-acid hydrazide **(2)** (2.51g, 0.01 mol) and substituted ketones (0.01 mol) in absolute ethanol was refluxed in water bath for 20 hr in presence of glacial acetic acid (1ml). The reaction mixture was cooled and poured into crushed ice. The product obtained was filtered, washed, dried and recrystallised from aqueous DMF.
Similarly various Schiff bases were prepared using different ketones.

TABLE-1:Physical data of Dibenzo[b,f]azepine-5-carboxylic acid[1-(substituted-phenyl)-ethylidene]-hydrazide (4a-j)

Compound	Substituents R	Molecular formula	Molecular Weight	Melting Point ^{0}C	Yield %	R_f value*
4a	2-OH	$C_{23}H_{19}O_2N_3$	369	204	88.8	0.60
4b	4-Cl	$C_{23}H_{18}ON_3Cl$	387.5	243	72.5	0.63
4c	4-NO_2	$C_{23}H_{18}O_3N_4$	398	219	99	0.59
4d	4 -Br	$C_{23}H_{18}ON_3Br$	432	208	89	0.67
4e	4 -OH	$C_{23}H_{19}O_2N_3$	369	215	73	0.56
4f	4-NH_2	$C_{23}H_{20}ON_4$	368	240	81	0.64
4g	4-CH_3	$C_{24}H_{21}ON_3$	367	209	74.5	0.55
4h	4-F	$C_{23}H_{18}ON_2F$	357	158	86	0.61
4i	H	$C_{23}H_{19}ON_3$	353	125	69	0.59
4j	4 -OCH_3	$C_{24}H_{21}O_2N_3$	383	213	90	0.53

**Cyclohexane:Ethylacetate (3:2).*

Dibenzo[b,f]azepine-5-carboxylic acid[1-(2-hydroxy-phenyl)-ethylidene]-hydrazide (4a)

IR (KBr) cm^{-1}: 3357 (N-H and O-H str), 3052, 3019 (ArC-H str), 1687 (C=O str), 1605 (C=N str), 1370 (C-N def).; ^{1}H-NMR ($CDCl_3$)δ: 13.02 (s, 1H, OH), 8.7 (s, 1H, NH), 7.9-6.6 (m, 12H, ArH), 6.07 (s, 2H, CH=CH), 1.93 (s, 3H, CH_3)

Dibenzo[b,f]azepine-5-carboxylic acid[1-(4-chloro-phenyl)-ethylidene]-hydrazide (4b)
Elemental analysis: Calc: C (71.22 %), H (4.64 %), N (10.83 %), Found C (70.30 %), H (4.51 %), N (9.92 %)

Dibenzo[b,f]azepine-5-carboxylic acid[1-(4-nitro-phenyl)-ethylidene]-hydrazide (4c)
IR (KBr) cm^{-1}: 3360 (N-H str), 3109 (ArC-H str), 1696 (C=O str), 1666 (C=N str), 1333 (C-N def), 1296 (Ar -NO_2 def); ^{1}H-NMR($CDCl_3$)δ: 8.9 (s, 1H, NH), 8.6-7.2 (m, 12H, ArH), 7.03 (s, 2H, CH=CH), 2 (s, 3H, CH_3).

Dibenzo[b,f]azepine-5-carboxylic acid[1-(4-amino-phenyl)-ethylidene]-hydrazide (4f)
IR (KBr) cm^{-1}: 3091, 3059 (NH_2 str), 3200 (N-Hstr), 2910, (ArC-H str), 1687 (C=O str), 1610 (C=N str), 1230 (C-N str).; ^{1}H-NMR (DMSO) **δ:** 9.93-9.42 (s, 2H, NH_2), 8.95-6.58 (m, 12 H, ArH), 6.06 (2H, CH=CH), 2.95 (s, 3H, CH_3).

Dibenzo[b,f]azepine-5-carboxylic acid[1-(4-fluoro-phenyl)-ethylidene]-hydrazide (4h)
IR (KBr) cm^{-1}: 3356 (N-Hstr), 2922 (ArC-H str), 1683 (C=O str), 1678 (C=N str), 1354 (C-N def), 1224.84 (Ar-F str).; ^{1}H-NMR (DMSO) δ: 9.6 (s, 1H, NH), 8.7-6.5 (m, 12 H, ArH), 6.07 (2H, CH=CH), 2.95 (s, 3H, CH_3).

Antibacterial Activity
The method followed was Agar Diffusion method [9,10]. The standard cultures of gram positive *Staphylococcus aureus* (ATCC 9144) and *Enterococcus fecalis* (ATCC 35550) and gram negative *Escherichia coli* (ATCC 25322), *Pseudomonas pneumoniae* (ATCC 15380) and *Salmonella typhi* (NCIM 2263) were used for determining the antibacterial activity of all the synthesized compounds.

TABLE-2: Antibacterial studies of Dibenzo[b,f]azepine-5 carboxylic acid[1-(substituted-phenyl)-ethylidene]-hydrazide (4a-j)

Compound	Substituents	Zone of inhibition (mm)				
	R	*E. coli*	*P.aureginosa*	*S.typhi*	*S.aureus*	*E. fecalis*
4a	2-OH	12	NI	NI	25	22
4b	4-Cl	15	NI	NI	26	NI
4c	4-NO_2	15	NI	NI	24	26
4d	4-Br	16	NI	NI	23	26
4e	4-OH	25	NI	NI	24	NI
4f	4-NH_2	22	NI	NI	23	NI
4g	4-CH_3	18	NI	22	NI	NI
4h	4-F	18	NI	NI	22	NI
4i	H	NI	NI	25	23	NI
4j	4-OCH_3	19	NI	NI	22	NI
Ampicillin		28	21	27	24	26

NI= no inhibition

The results are calculated as zone of inhibition in mm and tabulated (**Table-2).** From the antibacterial studies, it is seen that the compounds are more effective against gram positive than gram negative microorganisms. Almost all the title compounds have inhibited the growth of ***Staphylococcus aureus***, whereas they exhibited negligible activity against ***Pseudomonas aeuruginosa.*** Both electron donating and withdrawing substitutents have enhanced activity

against ***Staphylococcus aureus*.** Compounds **4g** and **4i** with *p*-methyl and acetophenone substitutents have shown good effects against ***Salmonella typhi*.** All the compounds have shown mild to moderate activity against ***E. coli.*** Compounds **4a, 4c** and **4d** with *o*-hydroxy, *p*-nitro and *p*-bromo substituents are effective against ***E. faecalis*.** However, none of the compounds have shown greater activity than the standard, Ampicillin (30 µg/ml).

Antitubercular Activity

The procedure involved the use of Middlebrook 7H-9 broth and standard strain of *M.tuberculosis* h37Rv [11]. The appearance of turbidity is considered as growth and indicates resistance to the compound. The standard drug used was Rifampicin (2.56µg/ml) and the results were expressed as MIC values **(Table-3)**

As seen from the table some of the compounds have shown good antitubercular activity as compared to the standard. Compounds **4b, 4d, 4h** and **4i** having *p*-chloro, *p*-bromo, *p*-fluoro and H are having the highest activity among the series with MIC value of 16 µg/ml. Compound **4c** with *p*-nitro substitution, has shown moderate activity with a MIC value of 32 µg/ml and rest of the derivatives in the series have shown MIC value at a very high concentration as compared to the standard. It is seen that derivatives with electron withdrawing substituents have shown higher activity than that with electron donating substituents. Among the electron withdrawing substituents, halogen substituted compounds have shown activity greater than nitro compounds. But none of the compounds have shown activity greater than the standard.

TABLE-3: Antitubercular studies of Dibenzo[b,f]azepine-5-carboxylic acid[1-(substituted-phenyl)-ethylidene]-hydrazide (4a-j)

Compound	Substituents R	MIC values (µg/ml) of Mycobacterium tuberculosis h37Rv
4a	2-OH	128
4b	4-Cl	16
4c	4-NO_2	32
4d	4-Br	16
4e	4-OH	64
4f	4-NH_2	64
4g	4-CH_3	128
4h	4-F	16
4i	H	16
4j	4-OCH_3	128

RESULTS AND DISCUSSION

The synthesis involves the acid-catalysed condensation of 5*H*-dibenzo (b,f)azepine-5*H*-acid hydrazide **(2)** with substituted aromatic ketones in ethyl alcohol containing catalytic amount of glacial acetic acid.

The intermediate 5*H*-dibenzo (b,f) azepine-5*H*- acid hydrazide **(2)** was obtained by condensing 5*H*-dibenzo (b,f) azepine-5*H*- carbonyl chloride and hydrazine hydrate in ethanol.

Purity of the compounds was checked by difference in m.p and R_f values. Formation of the products was confirmed by the IR spectra. The IR spectra showed the absence of NH_2 doublet, presence of C=O of amide at 1698.21 -1676cm^{-1}and formation of azomethine linkage (C=N str), at 1589-1605cm^{-1}. Further formation of the compounds 4a, 4c, 4f and 4h has been indicated by their ^{1}H NMR spectra showing aromatic proton at δ value of 8.95-6.61, NH singlet at 9.6-8.7 respectively. Satisfactory CHN analysis also authenticated the structure of the compounds.

These compounds were screened for antibacterial and antitubercular studies. Almost all the title compounds have inhibited the growth of *Staphylococcus aureus* where as they exhibited negligible activity against *Pseudomonas aeuruginosa.* Compounds 4g and 4i with *p*-methyl and acetophenone substitutents have shown good effects against *Salmonella typhi*. All the compounds have shown mild to moderate activity against *E. coli*. Compounds 4a, 4c and 4d with *o*-hydroxy, *p*-nitro and *p*-bromo substitutents are effective against *E. faecalis*. However, none of the compounds have shown activity greater than the standard, Ampicillin (30 µg/ml)

As seen from the table some of the compounds have shown good antitubercular activity as compared to the standard. Compounds 4b, 4d, 4h and 4i having *p*-chloro, *p*-bromo, *p*-fluoro and H are having the highest activity among the series with MIC value of 16 µg/ml. Compound 4c with *p*-nitro substituents has shown moderate activity with a MIC value of 32 µg/ml and rest of the derivatives in the series have shown MIC value at a very high concentration as compared to the standard.

CONCLUSION

The present work is focused on the synthesis of substituted phenyl carbonyl hydrazones of dibenzazepine. The activity is enhanced due to the presence of azomethine linkage. The structures of all the compounds were confirmed by IR, ^{1}H-NMR and CHN analysis.

Acknowledgement
The authors would like to thank Prof. B. G. Shivananda Principal, Al-Ameen College of Pharmacy, Bangalore, for providing support and necessary facilities. Authors also thank Astra Zeneca, Bangalore, and Quest Research & Training Institute, Bangalore for providing help in obtaining the various spectra and Sangenomics Research Labs Pvt Ltd Bangalore, for performing biological activities.

REFERENCES

[1] A A Jarrahpour, M Motamedifar, K Pakshir, N Hadi M. Zarei, *Molecules.*, **2004**, 9, 815-824.
[2] P.Rajput, S.S.Rajput, *Int. J. PharmTech Res.*, **2009,** 1(4), 1605-1611.
[3] Sie-Tiong Ha, Lay-Khoon Ong, Yip-Foo Win, Teck-Ming Koh , Guan-Yeow Yeap., *Molbank.*, **2008**, M582.
[4] J J Vora, S B Vasava, K C Parmar, S K Chauhan , S S Sharma, *E-J Chem.*, **2009** , 6 (4) , 1205-1210.
[5] B Narayana, B V Ashalatha , K K Vijaya Raj, N Suchetha Kumari., *Indian J Chem*, **2006**, 45B, 2696-2703.
[6] K G Desai, K R Desai, *Indian J Chem.*, **2005**, 44B, 2097- 2101.

[7] K K Prasad, M P Torakar, V M Kulkarni , V J Kadam, *Indian Drugs*, **2008**, 45(12), 934 - 939.
[8] K Mogilaiah, H Ramesh Babu, R Babu Rao , *Indian.J.Chem*, , **2001**, 40B, 1270 -74.
[9] J P R Cruikshank, B P Duguid, R H H S Marmion, *Medical Microbiology*, Churchill Livingstone, New York; Vol. **2** , **1995,**190.
[10] A Cremer. *Microbiological methods.* 6th ed. Butterworth and Co., London , **1991**, 235.
[11] J M Andrews, *J.Antimicrob. Chemother*., **2001**, 48, 5.