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Synthesis and biological activity of 2-[(2, 3-dimethyl phenyl) amino]benzonamide azetidine-2-ones

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ABSTRACT

The reaction of 2-[(2,3-dimethylphenyl) amino] ethyl benzoate **(1)** with hydrazine hydrate in dry benzene gave 2-[(2,3-dimethylphenyl) amino] benzocarbohydrazide **(2)**. Compound **(2)** upon treatment with substituted aromatic aldehydes to furnished the corresponding schiff's base **(3a-e)** which on treatment with acetyl chloride / with phenyl acetyl chloride and triethylamine in presence of dry benzene afforded 2-[2,3-dimethylphenyl amino)-N'-[2-oxo-4-(substituted phenyl) azetidine-1-yl]benzamide **(4a-e)** and 2-(2,3-dimethylphenyl amino)-N'-[2-oxo-3-phenyl-4-(substituted phenyl) azetidine-1-yl] benzamide **(5a-e)**. All newly synthesized compounds were characterised on the basis of IR, ^{1}HNMR and mass spectral data.

KEYWORDS

Mefananic acid;
Azetidinones;
Antimicrobial;
Antitubercular profile.

INTRODUCTION

The discovery of non-classical β-lactum antibiotics has attracted considerable attention of the synthetic organic chemists to explore and develop new mild and better yielding synthetic route to azetidin-2-ones[1-3]. Apart from the substructure of widely used antibiotics such as penicillin, cephalosporins, monobactams etc., the azetidine-2-one (β-lactum) skeleton has been recognized as a useful building block in streoselective synthesis of biologically important compounds[4,5]. Literature survey reveals that various azetidinones[6,12] have attracted considerable attention as they are also endowed with wide range of pharmaceutical activities. In light of these findings, synthesis of some novel azetidinones derivatives associated with mefanamic acid was converted to its hydrazide and the Schiff's bases **(3a-e)** obtained from the same were reacted with acetyl chloride / phenyl acetyl chloride to yield corresponding azetidine-2-ones 2-(2,3-dimethylphenyl amino)-N′-[2-oxo-4-(substituted phenyl) azetidine-1-yl] benzamide **(4a-e)** and 2-(2,3-dimethylphenyl amino)-N′-[2-oxo-3-phenyl-4-(substituted phenyl) azetidine-1-yl] benzamide **(5a-e)** respectively. The structures of these azetidin-2-ones were confirmed by their elemental analyses and spectral (IR, ^{1}HNMR) data.

EXPERIMENTAL

The reagents and solvents used for the synthesis were obtained commercially and further purified. The melting points were determined by open capillaries and are uncorrected.

Infra red spectra were recorded on an FTIR-8400

Shimadzu Spectrophotometer Department of Pharmaceutical Chemistry, Karnataka College of pharmacy, Bidar. The ^{1}HNMR spectra were recorded ACF 200 Supercon-Switzerland NMR Spectraphotometer. The chemical shifts were expressed in ppm (delta scale). Mass spectra were taken by using LC-MS 2010 (SHIMADZU) and the purity of the compounds was checked by TLC.

2-[(2,3-dimethylphenyl) amino]benzocarbohydrazide (2)

To a solution of 2-[(2,3-dimethylphenyl) amino] ethyl benzoate **(1)** (0.01mol) anhydrous alcohol (50ml), hydrazine hydrate 99% (0.3mol) and Conc. Sulphuric acid (6-8drops) were added. The reaction mixture was refluxed for 24h.The excess of solvent was distilled under reduced pressure and the mixture was poured on crushed ice with constant stirring. The solid separated was filtered, washed, dried and crystallized from ethanol. Yield 79%, m.p 170^{0}C, (Found C, 70.50, H, 6.67, N, 16.57 $C_{15}H_{17}N_3O$ requires C, 70.56, H, 6.71, N, 16.46 %).

2-[(2,3-dimetylphenyl) amino] -N′-(arylidene) bezohydrazide (3a-e) (General procedure)

To a solution of mefenamic acid hydrazide (0.01mole) and substituted aromatic aldehydes (0.01mole) was dissolved in dry benzene (30ml). A Dean stark water separator was attached and mixture was heated till the theoretical amount of water separated out, this was then concentrated under reduced pressure and the product obtained was crystallized from suitable solvent.

2-(2,3-dimethylphenyl amino)-N‘-[2-oxo-4-(substituted phenyl) azetidine-1-yl] benzamide (4a-e) (General procedure)

To a solution of **(3a-e)** (0.02mole) in dry benzene (30ml), few drops of triethyl amine and a solution of acid chloride (0.02mole) was added with stirring and refluxed for 2h. Triethyl amine hydrochloride formed was filtered off and washed 2-3 times with dry benzene. The filtrate and washing were concentrated under reduced pressure. The residue obtained was dried and crystallized from suitable solvent.

TABLE 1: Physical data of the synthesized compounds

Compd. no	R	Yield (%)	M.P (^{0}C)	Rf value	Molecular formula
(3a)	C_6H_5	60.50	216-218	0.61	$C_{22}H_{21}ON_3$
(3b)	C_6H_4OH(p)	65.25	218-220	0.68	$C_{22}H_{22}O_2N_3$
(3c)	C_6H_4Cl(p)	70.10	250-252	0.63	$C_{22}H_{21}ON_3Cl$
(3d)	C_6H_4Br(p)	66.20	208-210	0.64	$C_{22}H_{21}ON_3Br$
(3e)	$C_6H_4OCH_3$(p)	69.30	168-170	0.62	$C_{23}H_{24}O_2N_3$
(4a)	C_6H_5	70.70	203-205	0.80	$C_{24}H_{23}N_3O_2$
(4b)	C_6H_4OH(p)	61.45	148-150	0.71	$C_{24}H_{23}O_3N_3$
(4c)	C_6H_4Cl(p)	68.70	156-158	0.68	$C_{24}H_{22}O_2N_3Cl$
(4d)	C_6H_4Br(p)	65.35	123-125	0.79	$C_{24}H_{22}O_2N_3Br$
(4e)	$C_6H_4OCH_3$(p)	68.91	158-160	0.72	$C_{25}H_{25}O_5N_3$
(5a)	C_6H_5	69.55	128-130	0.79	$C_{30}H_{26}N_3O_2$
(5b)	C_6H_4OH(p)	66.61	198-200	0.69	$C_{30}H_{27}O_3N_3$
(5c)	C_6H_4Cl(p)	70.10	178-180	0.63	$C_{30}H_{26}O_2N_3Cl$
(5d)	C_6H_4Br(p)	69.12	118-120	0.65	$C_{30}H_{26}O_2N_3Br$
(5e)	$C_6H_4OCH_3$(p)	68.56	173-175	0.72	$C_{31}H_{29}O_3N_3$

***The compound gave satisfactory C, H and N analysis,*All the compounds are crystallized in ethanol.**

2-(2,3-dimethylphenyl amino)-N′-[2-oxo-3-phenyl-4-(substituted phenyl) azetidine-1-yl] benzamide (5a-e) (General procedure)

A mixture of **(3a-e)** (0.02mole) and phenyl acetyl chloride (0.02mole) in dry benzene (30ml), few drops of triethyl amine was added with stirring. The reaction mixture was refluxed for 2h. Triethyl amine hydro chloride formed was filtered off and washed 2-3 times with dry benzene. The filtrate and washing were concentrated under reduced pressure. The residue obtained was dried and crystallized from ethanol. The physical data of synthesized compounds were reported in TABLE 1.

RESULT AND DISCUSSION

Compounds synthesized during the present investigation were established on the basis of analytical, physical and spectral data as IR, ^{1}HNMR and mass spectra. 2-[(2,3-dimethylphenyl) amino] benzocarbo hydrazide **(2)** was confirmed on the basis of spectral data. The IR spectrum of **(2)** showed strong absorption band at 3326 cm^{-1} due to NH stretching, 1650 cm^{-1} due to C=O respectively. The ^{1}HNMR spectrum of **(2)** exhibited a two singlet at δ 2.0 and 2.2 corresponding six protons of two methyl groups and singlet at δ 4.1 two protons of NH_2 and multiplet at δ 6.5-7.0 corresponds to seven protons of aromatic ring and singlet at δ 7.3

TABLE 2: Antimicrobial activity of synthesized compounds

Compd. no	Zone of Inhibition in mm*					
	Antibacterial activity				Antifungal activity	
	E.coli	*P.aeruginosa*	*S.epidermatitis*	*B.subtilis*	*A.niger*	*C.albicans*
(3a)	10	12	12	14	10	12
(3b)	14	18	16	20	14	14
(3c)	12	18	12	18	14	16
(3d)	14	22	16	22	16	16
(3e)	12	18	12	16	12	10
(4a)	10	18	12	18	10	14
(4b)	14	16	16	20	14	10
(4c)	12	16	10	14	12	10
(4d)	10	16	12	18	14	14
(4e)	14	20	12	20	16	10
(5a)	10	18	10	22	16	12
(5b)	12	18	12	18	10	10
(5c)	14	22	14	20	12	14
(5d)	14	20	12	22	14	12
(5e)	16	24	16	24	18	18
Control(DMF)	6	6	6	6	6	6
Ciprofloxacin	24	25	24	25	--	--
Flucanazole	--	--	--	--	20	20

***Bore of the diameter 6mm, Standard: Ciprofloxacin and flucanazole**

and 8.0 corresponds to each proton of –NH and CO-NH. Further the structure of compound **(2)** is supported by mass spectrum show a molecular ion peak at m/z 256, which corresponds to its molecular formula. The elemental analysis of **(2)** shows (Found C, 70.50, H, 6.67, N, 16.57 $C_{15}H_{17}N_3O$ requires C, 70.56, H, 6.71, N, 16.46 %).

The reaction of 2-[(2,3-dimethylphenyl) amino] benzocarbohydrazide **(2)** was refluxed with P-bromo benzaldehyde in dry benzene and the reaction mixture was refluxed for about 6h. The separated solid was filtered and dried afforded the compound **(3d)** in good yield. The IR Spectrum of compound **(3d)** showed strong absorption band at 1650 cm^{-1} due to (CONH), and 1575 cm^{-1} due to (C=C), 1250 cm^{-1} due to (CO-C). Absence of the NH band of hydrazide in the spectrum of **(3d)** confirms the formation of Schiff's base. ^{1}HNMR spectrum of **(3d)** exhibited a two singlet at δ 2.0 and 2.2 due to six protons of two methyl groups and δ 6.5-7.8 corresponds to eleven protons of aromatic ring and singlet at δ 8.5 one proton of C-O-NH and singlet at δ 9.3 one proton of N=CH. The structure of compound **(3d)** is supported by mass spectrum show a molecular ion peak at m/z 422 which corresponds to its molecular formula $C_{22}H_{21}ON_3Br$ **(3d).** The spectral data of compounds **(3a-c,e)** are in agreement with the proposed structure **(3d)**.

Compound 2-[(2,3-dimetylphenyl) amino]-N'-(arylidene) bezohydrazide **(3a-e)** upon treatment acetyl chloride in presence of triethylamine and the reaction mixture was refluxed for 1h.The separated solid filtered and dried to gave **(4a-e)** in good yield. The IR Spectrum of Compound **(4d)** showed strong absorption band at 3344 cm^{-1} due to (N-H str), 1868 cm^{-1} due to (C=O of β-lactam), 1654 cm^{-1} due to (CONH), ^{1}HNMR spectrum of **(4d)** exhibited a two singlet at δ 1.9 and 2.3 corresponding six protons of two methyl groups and δ 2.7 doublet of two protons of azetidine-2-one C_3 and δ 3.3 singlet of one proton of azetidine-2-one C_4 and δ 7.2 singlet of one proton of Ph-NH-Ph and δ 6-8 multiplet of eleven protons of aromatic ring and δ 9.1 singlet of one proton of C-O-NH. Further the structure of compound **(4d)** is conformed by mass spectrum, which shows a molecular ion peak at m/z 464, which corresponds to its molecular formula $C_{24}H_{22}N_3O_2Br$ **(4d)**. The remaining compounds **(4a-c, e)** are agreed with spectral and analytical data.

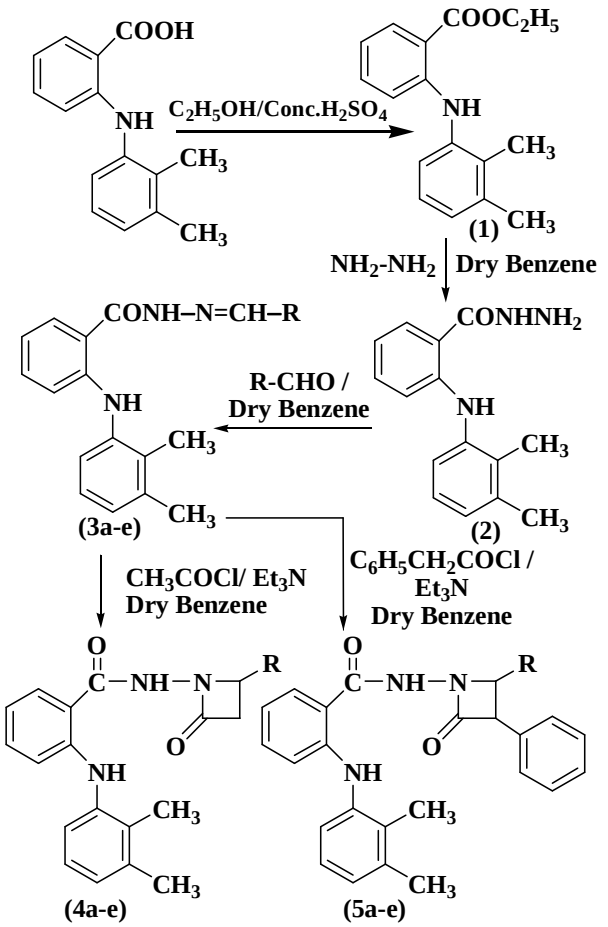
Compound **(3a-e)** upon treatment phenyl acetyl chloride in presence of triethylamine and the reaction mixture was heated for 1h. The separated solid filtered and dried furnished compounds **(5a-e)** in good yield. The IR Spectrum of Compound **(5d)** showed absorption band at 3342 cm^{-1} due to (N-H str), 1870 cm^{-1} due to (C=O of β-lactam), 1656 cm^{-1} due to (CONH).

Full Paper

TABLE 3: Antitubercular activity result of some selected compounds

Compound no.	Minimum inhibitory concentration	H37Rv concentration (μg/ml)
	125	250
Streptomycin (Standard)	S	250
4b	R	R
4c	S	S
4e	S	S
5b	R	R
5c	S	S
5e	S	S

S= Sensitive, R=Resistance



Where R: C_6H_5, C_6H_4(OH) (P), C_6H_4(Cl) (P), C_6H_4(Br)(p), $C_6H_4OCH_3$(P)

SCHEME 1

^{1}HNMR spectrum of **(5d)** exhibited a two singlet at δ 1.9 and 2.3 corresponding six protons of two methyl groups and δ 2.7 doublet of two protons of azetidine-2-one C_3 and δ 3.3 singlet of one proton of azetidine-2-one C_4 and δ 7.2 singlet of one proton of Ph-NH-Ph and δ 6-8 multiplet of eleven protons of aromatic ring and δ 9.1 singlet of one proton of C-O-NH. Further the structure of compound **(5d)** is conformed by mass spectrum, which shows a molecular ion peak at m/z 540, which corresponds to its molecular formula $C_{30}H_{26}N_3O_2Br$ **(5d)**. The remaining compounds **(5a-c, e)** are agreed with spectral and analytical data.

Evaluation of antimicrobial and antitubercular activity

Antibacterial and antifungal activity

The newly synthesized compounds were subjected to *invitro* antimicrobial activity against by Cup-Plate diffusion method[13] using organisms *E.coli, P.aeurginosa, S.epidermatitis, B.subtilis* for antibacterial activity where as *A.niger* and *C.albican* for antfungal activity. All the test compounds were prepared at the concentration of 100μg/ml in distilled DMF. The solution of ciprofloxacine and flucanazole were prepared at the concentration of 100μg/ml in sterile water as standard solution for compression of antibacterial and antifungal activities and DMF was used as control for both activity, the results were presented in TABLE 2.

The compounds **(3d, 4e, 5c** and **5e)** shown good antibacterial activity against *P.aeurginosa, B.subtilis* and remaining compounds are exhibited moderate activity against the *P.aeurginosa, B.subtilis.* In fungicidal activity the compounds **(3d, 4e, 5a** and **5e)** exhibited significant antifungal activity against *A.niger* and *C.albican* where as remaining compounds are exhibited moderate to weak activity against the *A.niger* and *C.albican* The results were presented in TABLE 2.

Antitubercular activity

Selected derivatives of azetidine-2-ones **(4b, 4c, 4e, 5b, 5c** and **5e)** were evaluated for their antitubercular activity against H37 Rv of *mycobacterium tuberculosis* Lowenstein Jensen's egg medium by serial two fold dilution method and the retardation of growth rate studied upto six weeks at 37^{0}C. The results were given in TABLE 3.

Compounds **(4c, 4e, 5c)** and **(5e)** were exhibited good antitubercular activity against *mycobacterium tuberculi* were as compound **(4d)** and **(5b)** showed less activity against strain of *mycobacterium tuberculi.*

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