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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF A NEW SERIES OF 2-[(PHENYLAMINO) ACETYL]-2,3-DIHYDROPHTHALAZINE-1,4-DIONE DERIVATIVES

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This research article presents the synthesis & antimicrobial activity of a new series of 2-[(phenylamino) acetyl]-2,3-dihydrophthalazine-1,4-dione derivatives **3**(a-f). The structures of the compounds were confirmed by FT-IR, ^{1}H NMR and Mass spectral data. Their antimicrobial activities were tested on 7 strains. All the derivatives have shown moderate to excellent antimicrobial activities. Compounds **3**a & **3**b have shown remarkable minimum inhibitory concentration especially against *Aspergillus niger* & compound **3**f against *Aspergillus flavus* when compared with the standard drugs.

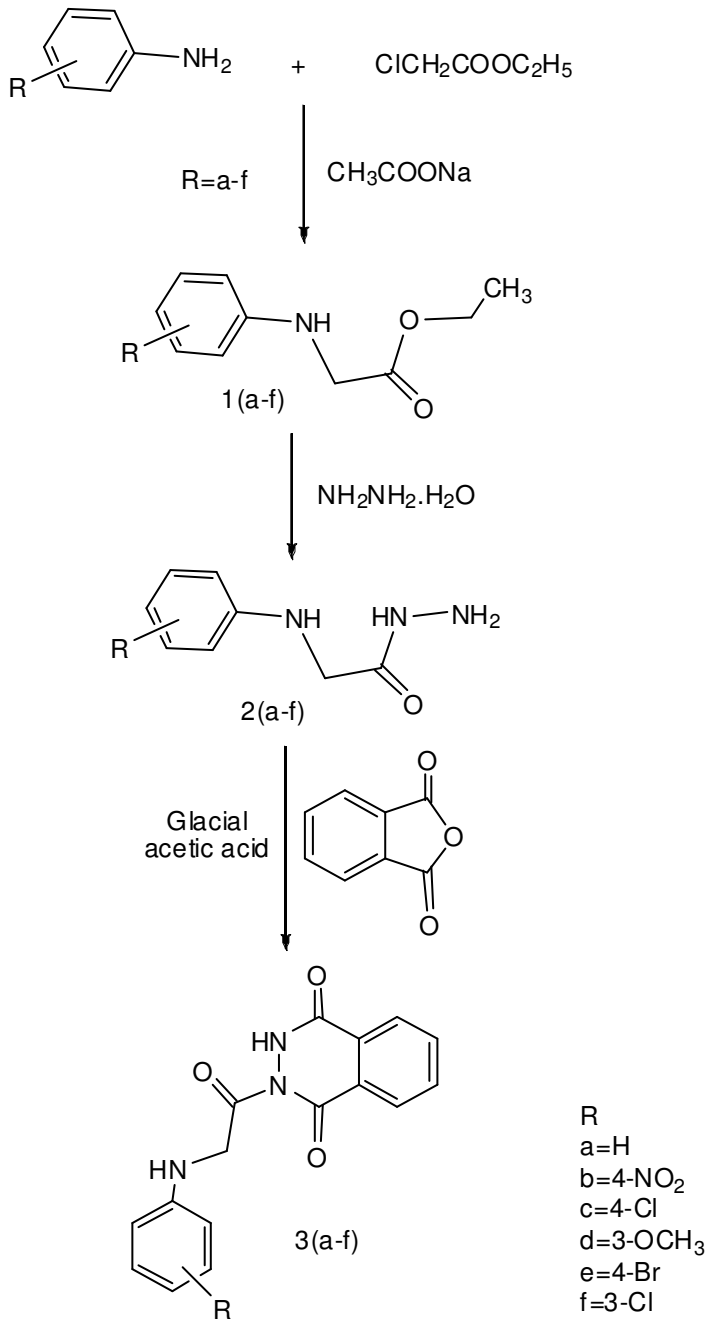
Azines have gained considerable importance in medicinal chemistry. Some of these compounds have presented antimycobacterial[1], cytotoxic[2], antimicrobial[3] and antipsychotic[4] properties. Azines have also been extensively used as ligands in coordination chemistry, since the flexibility of these compounds around the N-N bond offers different mononucleating and binucleating coordination modes. The diazine rings are building blocks of many important natural and synthetic compounds e.g. nucleotides. This is why, the simplest diazine, pyrazine, pyridazine, thiadiazine have early attracted attention of chemists as having antimycobacterial[5], antibacterial[6], anticonvulsant[7], anti-inflammatory analgesic[8], antitubercular, antiviral and anticancer[9] activities. Phthalazine, also called benzo-orthodiazine or benzopyridazine is a heterocyclicazine. Phthalazine moiety are of interest because they show some pharmacological and biological activities like antitumor[10] and antiinflammatory[11]. Further, carbonyl group attached to these azines or any other heterocyclic compound are said to be more efficacious like attachment of carbonyl group to phthalazines, benzoxazines results in phthalazinones & benzoxazinones respectively[12]. Phthalazin-1(2*H*)-ones are of considerable interest due to their antidiabetic[13], antiallergic[14], vasorelaxant[15], PDE4 inhibitors[16], VEGF (vascular endothelial growth factor) receptor tyrosinekinases for the treatment of cancer[17] and bronchodialation[18] and herbicidal[19] activities. A number of established drug molecules like Hydralazine[20], Budralazine[21], Azelastine[22], Ponalrestat[23] and Zopolrestat[24] are prepared from the corresponding phthalazinones.

Antimicrobial activity

In vitro antimicrobial activity was tested by disc diffusion method[25] and Broth Micro-Dilution method[26] using pathogenic strains of *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae* and

Table-1
Physical data of synthesized compounds 3(a-f)

Compd	R	M.P. (^{0}C)	Yield (%)
3a	H	168-70	67
3b	3-NO_2	238-40	68
3c	4-Cl	218-20	87
3d	3-OCH_3	60-80	75
3e	4-Br	224-26	82
3f	3-Cl	216-18	73



R
a=H
b=4-NO_2
c=4-Cl
d=3-OCH_3
e=4-Br
f=3-Cl

SCHEME-1

Table-2
Zone of inhibition (mm) values of the synthesized compounds 3(a-f)

Compd	Antibacterial				Antifungal		
	S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*	*C. albicans*	*A. niger*	*A. flavus*
3a	10	-	10	-	22	15	15
3b	12	-	12	-	20	10	12
3c	14	-	8	10	15	11	-
3d	10	-	8	10	15	-	12
3e	11	10	-	9	30	10	8
3f	12	11	-	8	14	-	10
Standard*	21	26	32	15	27	18	22

*Standard for antibacterial : Norfloxacin.

Standard for antifungal : Griseofulvin.

Table-3
MIC (µg/ml) values of the synthesized compounds 3(a-f)

Compd	Antibacterial				Antifungal		
	S. aureus	*B. subtilis*	*K. pneumoniae*	*E. coli*	*C. albicans*	*A. niger*	*A. flavus*
3a	100	100	100	100	50	0.4	6.25
3b	100	12.5	100	100	50	0.4	6.25
3c	100	50	100	100	100	0.4	12.5
3d	100	50	12.5	100	50	0.8	25
3e	100	100	100	100	100	1.6	25
3f	100	100	100	100	125	12.5	0.4
Standard (1)	2	2	1	2	16	8	8
Standard (2)	3	1	1	12	500	100	100

Standard (1) for antibacterial : Ciprofloxacin
Standard (2) for antibacterial : Norfloxacin
Standard (1) for antifungal : Fluconazole
Standard (2) for antifungal : Griseofulvin

Escherichia coli. The fungi used were *Candida albicans, Aspergillus niger* and *Aspergillus flavus*. Known antibiotic Ciprofloxacin and Norfloxacin were used as standard drug for antibacterial activity and

Fluconazole and Griseofulvin were used as standard drugs for antifungal activity. The experimental results of antibacterial and antifungal activity indicated a variable degree of efficacy of the compounds against different strains of bacteria & fungi and are depicted in Table-2 & 3. Compounds **3**a and **4**b have shown remarkable Minimum Inhibitory Concentration especially against *Aspergillus niger* & compound **3**f against *Aspergillus flavus* when compared with the standard drugs.

Experimental

Melting points of the synthesized compounds were determined on SHITAL-Digital Programmable melting point apparatus and are uncorrected: FT-IR spectra were recorded on Bruker spectrophotometer by using KBr pellets. The ^{1}H NMR spectra were recorded on a Bruker Avance III NMR 300 MHz instruments using DMSO as solvent and TMS as internal standard (chemical shifts in δ) and the homogeneity of the compounds was determined by TLC on silica gel G plates and spots were visualized in iodine vapour. The physical properties of the synthesized compounds are depicted in Table-1.

Preparation of ethyl 2-(substituted phenylamino) acetate[27] 1(a-f)

A mixture of substituted anilines (0.1 mmol), ethyl chloroacetate (0.12 mmol), anhyd sodium acetate (0.15 mmol) in 50 ml absolute ethanol was refluxed for 6 hr. The mixture was cooled and kept overnight at room temp. It was poured into cold water. The solid obtained was washed with cold water.

Preparation of hydrazides[27] 2(a-f)

Hydrazine hydrate (80%, 0.1 mmol) was added dropwise to ethyl 2-(substituted phenylamino) acetates (**1**a-f) (0.1 mmol) with constant stirring. The mixture was gently refluxed for 15 min. Then about 25 ml absolute ethanol was added to produce clear solution. The reaction mixture was refluxed for 3 hr. The ethanol was distilled off under reduced pressure and cooled. Resulting solid was recrystallized form ethanol.

Preparation of 2-(2-(phenylamino) acetyl) 2-3-dihydrophthalazine-1,4-dione[28] (3a-f)

A mixture of substituted hydrazides (**2**a-f) (0.01 mol) and phthalic anhydride (0.01 mol) in absolute ethanol and glacial acetic acid (0.005 mol) was refluxed for 3 hr and cooled. The reaction mixture was poured into crushed ice. The solid obtained was filtered washed with dilute sodium bicarbonate solution and recrystallized from ethanol.

Spectral data

3a : IR (KBr cm^{-1}): 3328 (-NH str), 3047 (Aromatic -CH str), 2914 (aliphatic -CH str), 1747 (C=O str), ^{1}H NMR (δ ppm): 10.64 (s, 1H of NH), 7.91-7.80 (m, 4H, ArH of phthalazine), 7.15-7.06 (m, 2H of aromatic ring) , 6.68-6.58 (m, 3H of aromatic ring), 5.80 (t, 1H, NH), 3.98, 3.95 (d, 2H of CH_2).

3b : IR (KBr): 3304 (-NH str), 3130 (aromatic -CH str), 2923 (aliphatic -CH str), 1709 (C=O str), 1533 (asym, -NO_2 str), 1350 (sym, -NO_2 str), ^{1}H NMR : 10.70 (s, 1H of NH), 7.99-7.94 (m, 2H, ArH of phthalazine), 7.91-7.89 (d, 2H, ArH of phthalazine), 7.58-7.50 (m, 4H of aromatic ring), 3.54, 3.52 (d, 2H of CH_2).

3c: IR (KBr): 3330 (-NH str), 2925 (aliphatic -CH str), 1745 (C=O str), 1227 (C-Cl bend), ^{1}H NMR : 10.79 (s, 1H of NH), 7.97-7.92 (m, 4H, ArH of phthalazine), 7.15-7.14 (dd, 2H of aromatic ring), 6.66-6.64 (dd, 2H of aromatic ring), 6.37, 6.36, 6.35 (t, 1H of NH), 3.97-3.95 (d, 2H of CH_2). Mass : m/z 328 (M-1).

3d: IR (KBr): 3508 (-NH str), 3182 (aromatic -CH str), 2970 (aliphatic -CH str), 1737 (C=O str), 1047, ^{1}H NMR : 10.53 (s, 1H of NH), 7.88-7.74 (m, 4H, ArH of phthalazine), 7.07-6.98 (m, 2H of aromatic ring), 6.27, 6.25 (d, 2H of benzene), 5.50 (s, 1H of NH), 3.81-3.77 (d, 3H of OCH_3), 3.74, 3.66 (d, 2H of CH_2).

3e : IR (KBr): 3330 (-NH str), 2922 (aromatic -CH str), 2884 (aliphatic -CH str), 1744 (C=O str), 1177,

1073 (C-Br bend). ^{1}H NMR : 10.66 (s, 1H of NH), 7.94-7.84 (m, 4H, ArH of phthalazine), 7.23, 7.21 (d, 2H of aromatic ring), 6.62, 6.60 (d, 2H of benzene), 3.96 (s, 2H of CH_2). Mass : m/z 372 (M-2).

3f: IR (KBr): 3317 (-NH str), 2913 (aromatic -CH str), 1735 (C=O str), 1217 (C-Cl bend). ^{1}H NMR : 10.71 (s, 1H of NH), 7.91-7.85 (m, 4H, ArH of phthalazine), 7.10, 7.08, 7.06 (t, 1H of aromatic ring), 6.66, 6.65 (t, 1H of benzene), 6.61-6.56 (m, 2H of aromatic ring, 3.33, 3.30 (d, 2H of CH_2)). Mass : m/z 328 (M-1).

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