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Synthesis of (E)-2-(arylbenzylidene)-2-((4-methoxyphenyl)amino) acetohydrazide derivatives and their antimicrobial activity

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A B S T R A C T

A series of some new (E)-2-(arylbenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazides **4(a-j)** have been conveniently synthesized by intermolecular oxidative cyclization of (E)-2-(arylbenzylidene)-2-[(4-methoxyphenyl)amino]acetohydrazides promoted by iodobenzene diacetate as an oxidant. The structures of the synthesized compounds have been confirmed by ^{1}H NMR, IR, MS and elemental analysis. All the synthesized compounds were tested for their inhibitory action against clinically isolated strains i.e., *Bacillus subtilis, Staphylococcus aureus, Xanthomonas campestris, Escherichia coli* and *Fusarium oxysporum*. Compounds **4f** (21-28 mm, 73.8 %), **4i** (20-24 mm, 72.4 %) and **4j** (21-27 mm, 71.6 %) demonstrated good antimicrobial activity against all the tested bacterial and fungal strains.

1. Introduction

In the past decades, the problem of multidrug resistant micro-organism has reached on alarming level around the world, and the synthesis of new anti-infective compounds has become an urgent need for the treatment of microbial infections. There are various problems arising with the use of antimicrobials such as local tissue irritation, interference with wound healing process, hypersensitivity reactions, system toxicity, narrow antimicrobial spectrum, and emergence of

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resistance.[1,2] Therefore, the increasing clinical importance of drug resistant microbial pathogens has additional urgency in microbiological and antifungal research. Compounds containing heterocyclic ring systems continue to attract considerable interest due to the wide range of biological activities they possess.[3] Among them five membered rings gained importace because of their versatile biological properties.[3] In particular, compounds bearing 1,3,4-oxadiazoles nucleus are known to have unique angioedema and anti-inflammatory activities.[4] Substituted oxadiazoles molecules possess other interesting properties such as analgesic[5], antimicrobial[6], antiviral[7], anticonvulsant[8], antihypertensive[9], anti-proliferative[10], enzyme Inhibitors[11], 5-HT-receptor antagonists[12] and inhibitors of muscle glycogen phosphorylase[13].

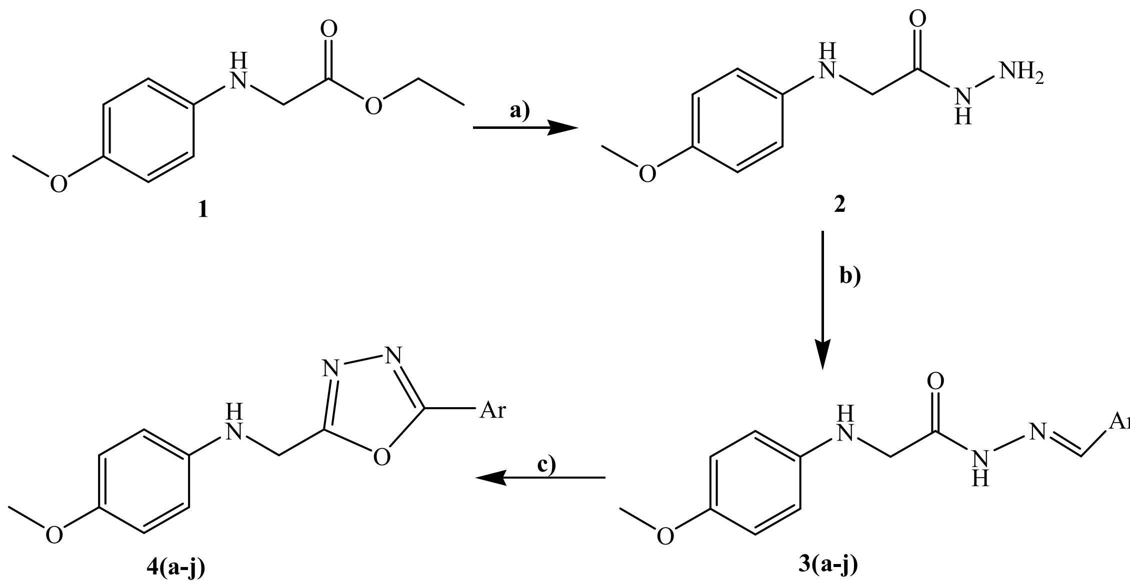
Literature reveals that docking study of *N*-(hetarylmethyl)-aniline derivatives showed anticancer activity[14] and *N*-aryl-*N*-benzylamines were synthesized and evaluated for their antifungal activity, which was compared with their homoallylamine analogues that possessed an allyl group in the carbon next to the nitrogen atom. Results indicated that the absence of the allyl group caused an enhancement of the antifungal activity which could be correlated with the flexibility of the alkyl chain between both aromatic groups.[15] This led to the preparation of *N*-substituted amines bearing a hetaryl fragments (*N*-(hetarylmethyl)-anilines).

2. Results and discussion

2.1. Chemistry

The new compounds were prepared by using the synthetic strategy described in **Scheme 1**. The 2-(4-methoxyphenylamino)acetohydrazide **2** was synthesized by the reaction of ethyl-(4-methoxyphenyl)glycinate **1** with hydrazine hydrate in ethanol as per the reported procedure[15].

The (*E*)-2-(arylbenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazide (**3a-j**) was synthesized by the reaction of 2-(4-methoxyphenylamino)acetohydrazide **2**. It was refluxed with different substituted aldehydes in ethanolic solution for about 2 h. A series of new 2,5-disubstituted-1,3,4-oxadiazoles (**Table 1**) have been accomplished in excellent yields by the oxidation of **3a-j** of various aryl aldehydes with one equivalent of iodobenzenediacetate (IBD) in dichloromethane. Compounds **3a-j** and **4a-j** were synthesized based on the reported procedure.[16, 17]

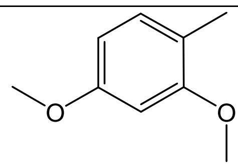
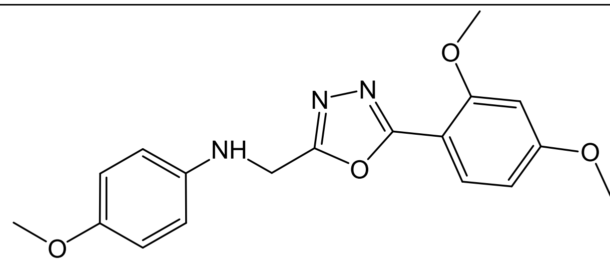
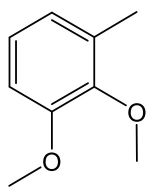
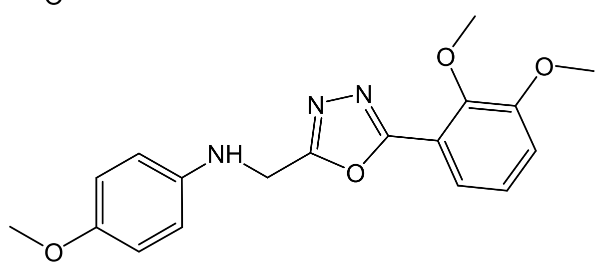
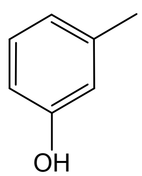
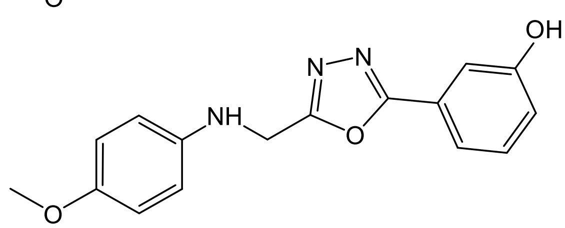
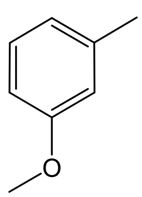
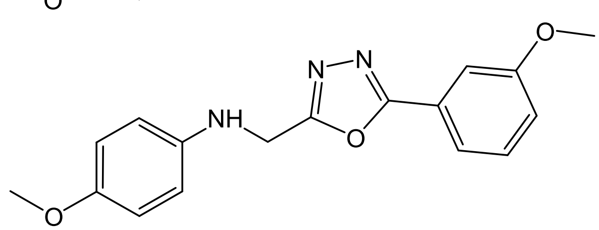
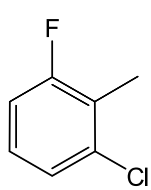
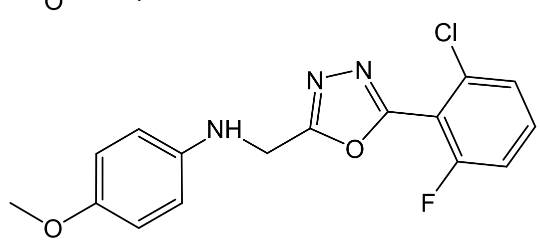


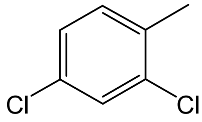
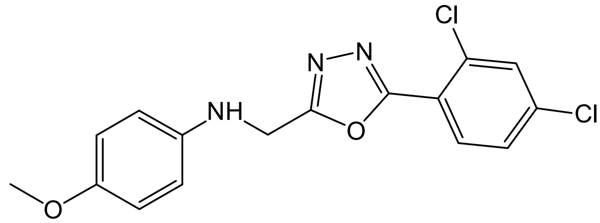
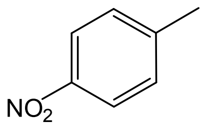
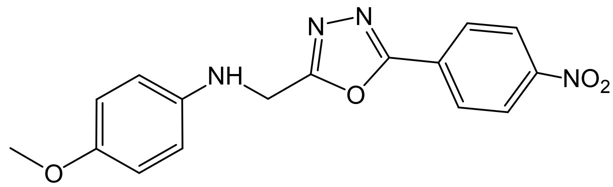
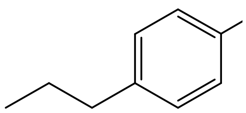
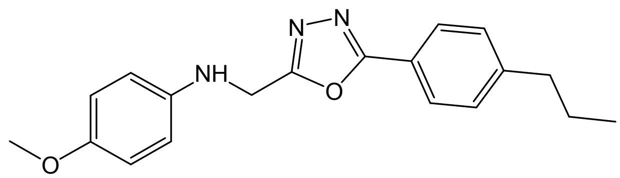
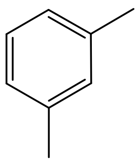
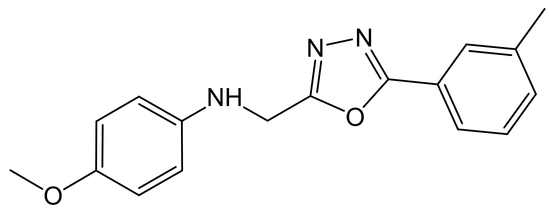
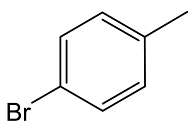
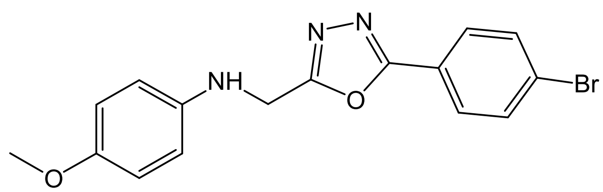
a) Hydrazine hydrate, ethanol, reflux 3 h, b) Aryl aldehydes, ethanol, reflux 2 h, c) IBD, DCM, reflux 2 h.

Scheme 1. Synthesis of compounds **4**

The IR spectra of the compound **3a** exhibited characteristic bands at absorption bands at 1631 cm^{-1} and 3150 cm^{-1} due to for carbonyl and NH group, respectively. The ^{1}H NMR spectra of **3a** showed two singlet due at N=CH and NH at δ 8.58 and δ 9.34, respectively. The structure of all compounds **4a-j** confirmed by their spectral (IR, LCMS, and ^{1}H NMR) and elemental analysis. The characterization of products **4a-j** was based upon a careful comparison of their IR and ^{1}H NMR spectra with those of **3a-j**. IR spectra of **4a** were found to be transparent in the region of NH stretch and CO stretch. In ^{1}H NMR spectra of **4a** the disappearance of their singlet due to N=CH around δ 8.4-8.6 and NH proton around δ 9.3-9.5, thus confirming the oxidation of **3a-j** into **4a-j**. The mass spectra of **4a** showed molecular ion peak at m/z 342, which is in agreement with the molecular formula $C_{18}H_{19}N_3O_4$.

Table 1. Chemical structure and physical data of 2,5-disubstituted-1,3,4-oxadiazoles (**4a-j**)

Comp.	Ar	Structure	Yield, %	mp (°C)
4a			80.0	126-128
4b			81.0	137-139
4c			76.0	144-146
4d			85.0	148-150
4e			77.0	150-152

4f			84.0	167-169
4g			86.0	171-173
4h			82.0	152-154
4i			79.0	146-148
4j			86.0	170-172

3a-j (1 eq), IBD (1 eq), methanol, reflux.

2.2. Antimicrobial activity

Compounds (**4a-j**) were tested *in vitro* for their antibacterial activity against two gram positive and two gram negative bacterial strains. Commercial antibiotics such as bacteriomycin and gentamycin were used as standard drugs. The results were compared with standard drugs and depicted in **Table 2**. Compound **4f** was found to be more potent against gram positive and gram negative bacterial strains with the zone of inhibition respectively 21-28 mm. Compounds **4i** and **4j** exhibited 20-24 mm and 21-27 mm, respectively against all the bacterial strains. Compounds **4a**, **4b,** **4d**, **4e** and **4g** were showed moderate antibacterial activity and compound **4d** was found to be slightly active than **4a**, **4e**, **4b** and **4g**. A compound **4h** was weakly active against tested bacterial strains.

The *in vitro* antifungal activity of the new oxadiazole derivatives (**4a-j**) was studied against the fungal strain, *Fusarium oxysporum*. Nystatin was used as a standard drug and the results are given in **Table 2**. Compounds **4f**, **4i** and **4j** showed 73.8 %, 72.4 % and 71.6 % inhibition, respectively when compared to standard drug (100 %). Compounds **4d** (69.0 %), **4e** (63.1 %), **4b** (56.4 %), **4g** (52.0 %) and **4a** (53.8 %) exhibited moderate antifungal activity against tested fungal strain. On the other hand, the lowest antifungal effect was detected for compounds **4h** (51.9 %) and **4c** (50.2 %) against tested fungal strain. In the present study different groups attached to aryl ring as substituent linkage to 1,3,4-oxadiazole ring. The close survey of antimicrobial efficacy indicated that the inhibition values of all the compounds exhibited a different range of activity against the tested strains. The biological results for compounds (**4a-j**) showed that the substitution pattern on phenyl ring appears to be vital for broad spectrum activity.

Table 2. *In vitro* antibacterial and antifungal activities of the synthesized compounds

Compound	Zone of inhibition in diameter (mm)				% Inhibition
	B. subtilis	*S. aureus*	*X. campestris*	*E. coli*	*F. oxysporum*
4a	18	18	17	16	53.8
4b	18	19	19	22	56.4
4c	12	13	12	13	50.2
4d	21	20	19	23	69.0
4e	19	19	20	21	63.1
4f	25	23	21	28	73.8
4g	16	18	17	16	52.0
4h	14	15	14	15	51.9
4i	22	21	20	24	72.4
4j	23	22	21	27	71.6
Bacteriomycin	-	-	34	-	-
Gentamycin	35	30	-	35	-
Nystatin	-	-	-	-	100

Antibacterial activity was expressed in Zone of inhibition (mm), Antifungal activity was expressed in % inhibition (mm)

3. Conclusions

In conclusion, a series of new 2,5-disubstituted-1,3,4-oxadiazoles (**4a-j**) were synthesized in good yield, characterized by different spectral studies and their antimicrobial activities have been evaluated. Compounds **4f**, **4i** and **4j** demonstrated good inhibition against microbial strains tested. The substituent on phenyl ring is responsible for the antimicrobial activity of these classes of agents. On the basis of their activity, these derivatives were identified as viable leads for further studies.

Acknowledgments

One of the authors (B. N. Prasanna Kumar) grateful to Synzene International Private Limited, Bangalore, India, given an opportunity to continue the higher education.

4. Experimental

4.1. Materials and Methods

All solvents and reagents were purchased from Merck Chemicals, India. Melting point was determined by VMP III apparatus. An elemental analysis was recorded on Vario MICRO superuser V1.3.2 Elementar. The IR spectra were recorded using KBr discs on IR Jasco 4100 infrared spectrophotometer. ^{1}H NMR spectra was recorded using d_6-DMSO as solvent on Bruker DRX-500 spectrometer at 400 MHz. The mass spectrum was recorded using the instrument LC-MSD-Trap-XCT.

*4.2. Synthesis of 2-(4-methoxyphenylamino)acetohydrazide **(2)***

A solution of ethyl-(4-methoxyphenyl)glycinate **1** (10 gm, 0.048 mmol) in ethanol was taken, hydrazine hydrate (5 mL) was added, and then reaction mixture refluxed for 3 h. The reaction mixture was cooled and the solid formed was filtered and washed with chilled ethanol. Yield: 78 %. mp 116-118 ^{o}C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 4.51 (s, 2H, NH_2), 4.60 (s, 2H, -CH_2), 7.10 (dd, 2H, Ar-H), 7.79 (dd, 2H, Ar-H).

*4.3. General procedure for the synthesis of (E)-2-(arylbenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazides **(3a-j)***

Compound **2** was refluxed with different aryl aldehydes in ethanolic solution (10 mL) for about 2 h. Reaction completion was confirmed by the thin layer chromatography (TLC). The reaction mass was cooled to 5 to 10 ^{o}C for 1 h. The reaction mass was filtered and washed with ethanol. The obtained solid was dried to get the pure product.

4.3.1. (E)-N-(2,4-Dimethoxybenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazide ***(3a)***

The compound **3a** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 2,4-dimethoxybenzaldehyde (0.85 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 90 %. mp 151-153 °C. IR (KBr, cm^{-1}): 3300 and 3150 (N-H), 3073 (C-H), 1681 (C=N), 1631 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.87 (s, 3H, -OCH_3), 3.88 (s, 6H, -OCH_3), 4.60 (d, 2H, -CH_2), 6.35 (s, NH), 7.03 (d, 2H, Ar-H), 7.44-7.48 (m, 3H, Ar-H), 7.82 (d, 2H, Ar-H), 8.58 (s, N=CH), 9.34 (s, NH).

4.3.2. (E)-N-(2,3-Dimethoxybenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazide ***(3b)***

The compound **3b** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 2,3-dimethoxybenzaldehyde (0.85 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 84 %. mp 155-157 °C. IR (KBr, cm^{-1}): 3310 and 3155 (N-H), 3070 (C-H), 1680 (C=N), 1634 (C=O), ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.87 (s, 6H, -OCH_3), 3.88 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.37 (s, NH), 7.16-7.20 (m, 4H, Ar-H), 7.88-7.90 (m, 3H, Ar-H), 8.57 (s, N=CH), 9.28 (s, NH).

4.3.3. (E)-N-(3-hydroxybenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazide ***(3c)***

The compound **3c** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 3-hydroxybenzaldehyde (0.63 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 88 %. mp 160-162 °C. IR (KBr, cm^{-1}): 3314 and 3171 (N-H), 3066 (C-H), 1676 (C=N), 1630 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.87 (s, 3H, -OCH_3), 4.58 (d, 2H, -CH_2), 5.22 (s, 1H, OH), 6.38 (s, NH), 7.01 (dd, 2H, Ar-H), 7.23-7.36 (m, 4H, Ar-H), 7.89 (t, 2H, Ar-H), 8.50 (s, N=CH), 9.20 (s, NH).

4.3.4. (E)-N-(3-methoxybenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazide ***(3d)***

The compound **3d** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 3-methoxybenzaldehyde (0.70 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 90 %. mp 171-173 °C. IR (KBr, cm^{-1}): 3318 and 3170 (N-H), 3069 (C-H), 1670 (C=N), 1630 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.87 (s, 3H, -OCH_3), 3.88 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.37 (s, NH), 7.04-7.10 (d, 4H, Ar-H), 7.59 (s, 1H, Ar-H), 7.66-7.75 (m, 3H, Ar-H). 8.51 (s, N=CH), 9.32 (s, NH).

4.3.5. (E)-N-(2-chloro-6-fluorobenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazide ***(3e)***

The compound **3e** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 2-chloro-6-fluorobenzaldehyde (0.81 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 84 %. mp 149-151 °C. IR (KBr, cm^{-1}): 3306 and 3170 (N-H), 3060 (C-H), 1672 (C=N), 1636 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.88 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.38 (s, NH), 7.08 (dd, 2H, Ar-H), 7.31-7.34 (t, 1H, Ar-H), 7.45-7.48 (m, 4H, Ar-H), 8.48 (s, N=CH), 9.29 (s, NH).

4.3.6. (E)-N-(2,4-dichlorobenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazide ***(3f)***

The compound **3f** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 2,4-dichlorobenzaldehyde (0.90 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 90 %. mp 164-166 °C. IR (KBr, cm^{-1}): 3308 and 3166 (N-H), 3069 (C-H), 1676 (C=N), 1630 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.88 (s, 6H, -OCH_3), 4.60 (d, 2H, -CH_2), 6.37 (s, NH), 6.99-7.10 (m, 8.26 Hz, 3H, Ar-H), 7.80-7.93 (m, 4H, Ar-H), 8.44 (s, N=CH), 9.33 (s, NH).

4.3.7. (E)-2-((4-methoxyphenyl)amino)-N-(4-nitrobenzylidene)acetohydrazide ***(3g)***

The compound **3g** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 4-nitrobenzaldehyde (0.77 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 89 %. mp 180-182 °C. IR (KBr, cm^{-1}): 3310 and 3170 (N-H), 3074 (C-H), 1680 (C=N), 1635 (C=O). ^{1}H

NMR (DMSO-d_6, 400 MHz) δ: 3.87 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.37 (s, NH), 7.05 (dd, 2H, Ar-H), 7.66 (dd, 2H, Ar-H), 8.04-8.13 (m, 4H, Ar-H). 8.43 (s, N=CH), 9.31 (s, NH).

*4.3.8. (E)-N-(4-propylbenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazide **(3h)***

The compound **3h** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 4-propylbenzaldehyde (0.76 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 90 %, mp 179-181 °C. IR (KBr, cm^{-1}): 3300 and 3165 (N-H), 3070 (C-H), 1678 (C=N), 1634 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.90 (t, 3H, CH_3), 1.54-1.62 (m, 2H, CH_2), 2.48-2.50 (t, 2H, CH_2), 3.87 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.37 (s, NH), 7.08 (dd, 2H, Ar-H), 7.77-7.52 (m, 4H, Ar-H), 7.90 (d, 2H, Ar-H). 8.41 (s, N=CH), 9.36 (s, NH).

*4.3.9. (E)-2-[(4-methoxyphenyl)amino]-N-((3-methylbenzylidene)acetohydrazide **(3i)***

The compound **3i** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 3-methylbenzaldehyde (0.62 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 85 %. mp 158-160 °C. IR (KBr, cm^{-1}): 3310 and 3168 (N-H), 3075 (C-H), 1671 (C=N), 1632 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.20 (s, 3H, -CH_3), 3.88 (s, 3H, -OCH_3), 4.60 (d, 2H, -CH_2), 6.36 (s, NH), 7.06 (dd, 2H, Ar-H), 7.77-7.84 (m, 5H, Ar-H), 8.10-8.15 (m, 1H, Ar-H). 8.41 (s, N=CH), 9.30 (s, NH).

*4.3.10. (E)-N-(4-bromobenzylidene)-2-[(4-methoxyphenyl)amino]acetohydrazide **(3j)***

The compound **3j** was obtained by the reaction of **2** (1.0 gm, 0.005 mmol) and 4-bromolbenzaldehyde (0.95 gm, 0.005 mmol) using the general procedure described for **3(a-j)**. Yield 88 %. mp 177-179 °C. IR (KBr, cm^{-1}): 3308 and 3168 (N-H), 3068 (C-H), 1672 (C=N), 1638 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.88 (s, 3H, -OCH_3), 4.60 (d, 2H, -CH_2), 6.36 (s, NH), 7.06-7.18 (m, 4H, Ar-H), 7.46 (d, 2H, Ar-H), 8.1 (d, 2H, Ar-H), 8.47 (s, N=CH), 9.32 (s, NH).

*4.4. General procedure for the synthesis of 2,5-disubstituted-1,3,4-oxadiazoles **(4a-j)***

(*E*)-2-(arylbenzylidene)-2-((4-methoxyphenyl)amino)acetohydrazides (**3a-j**) was dissolved in dichloromethane and IBD were added to it. The contents were stirred for 2 h and the progress of the reaction was monitored by TLC. The solvent was removed and the residue was taken in petroleum ether and stirred for 30 min. The solid thus obtained was filtered, washed with petroleum ether and dried to afford (**4a-j**).

*4.4.1. N-((5-(2,4-Dimethoxyphenyl)-1,3,4-oxadiazole-2-yl)methyl)-4-methoxyaniline **(4a)***

The compound **4a** was obtained by the reaction of **3a** (1.0 gm, 0.003 mmol) and iodobenzene diacetate (1.12 gm, 0.0034 mmol) using the general procedure described for **4(a-j)**. White solid**.** FT-IR (KBr, cm^{-1}): 3060 (aromatic C-H), 2929 (C-H of CH_2), 1680 (C=N), 1464 (C=C), 1375 (C-N), 1255 (C-O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.88 (s, 3H, -OCH_3), 3.89 (s, 6H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.34 (s, 1H, NH), 7.09 (d, 2H, Ar-H), 7.50-7.58 (m, 3H, Ar-H), 7.92 (d, 2H, Ar-H). MS (ESI) *m/z*: 342 (Expected mass: 341). Anal. Calcd. for $C_{18}H_{19}N_3O_4$ (in %): C, 63.33; H, 5.61; N, 12.31. Found: C, 63.25; H, 5.60; N, 12.25.

*4.4.2. N-((5-(2,3-Dimethoxyphenyl)-1,3,4-oxadiazole-2-yl)methyl)-4-methoxyaniline **(4b)***

The compound **4b** was obtained by the reaction of **3b** (1.0 gm, 0.003 mmol) and iodobenzene diacetate (1.12 gm, 0.0034 mmol) using the general procedure described for **4(a-j)**. White solid. FT-IR (KBr, cm^{-1}): 3070 (aromatic C-H), 2936 (C-H of CH_2), 1672 (C=N), 1470 (C=C), 1384 (C-N), 1243 (C-O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.88 (s, 6H, -OCH_3), 3.89 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.38 (s, 1H, NH), 7.08 (d, 2H, Ar-H), 7.16-7.20 (d, 2H, Ar-H), 7.85-7.88 (m, 3H, Ar-H). MS (ESI) *m/z*: 342 (Expected mass: 341). Anal. Calcd. for $C_{18}H_{19}N_3O_4$ (in %): C, 63.33; H, 5.61; N, 12.31. Found: C, 63.22; H, 5.58; N, 12.25.

*4.4.3. 3-(5-((4-Methoxyphenyl)amino)methyl)-1,3,4-oxadiazole-2-yl)phenol **(4c)***

The compound **4c** was obtained by the reaction of **3c** (1.0 gm, 0.0033mmol) and iodobenzene diacetate (1.29 gm, 0.004 mmol) using the general procedure described for **4(a-j)**. Brown solid**.** FT-IR (KBr, cm^{-1}): 3515 (OH), 3068 (aromatic C-H), 2930 (C-H of CH_2), 1652 (C=N), 1469 (C=C), 1388 (C-N), 1250 (C-O). ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.86 (s, 3H, -OCH_3), 4.60 (d, 2H, -CH_2), 5.20 (s, 1H, OH), 6.35 (s, 1H, NH), 6.88 (dd, 2H, Ar-H), 7.22-7.31 (m, 2H, Ar-H), 7.55 (s, 1H, Ar-H), 7.88 (d, 2H, Ar-H). MS (ESI) *m/z*: 298 (Expected mass: 297). Anal. Calcd. for $C_{16}H_{15}N_3O_3$ (in %): C, 64.64; H, 5.09; N, 14.13. Found: C, 64.42; H, 4.89; N, 14.10.

*4.4.4. 4-Methoxy-N-((5-(3-methoxyphenyl)-1,3,4-oxadiazole-2-yl)methyl]aniline **(4d)***

The compound **4d** was obtained by the reaction of **3d** (1.0 gm, 0.0032 mmol) and iodobenzene diacetate (1.23 gm, 0.0038 mmol) using the general procedure described for **4(a-j)**. White solid. FT-IR (KBr, cm^{-1}): 3060 (aromatic C-H), 2944 (C-H of CH_2), 1628 (C=N), 1430 (C=C), 1358 (C-N), 1247 (C-O). ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.88 (s, 6H, -OCH_3), 4.58 (d, 2H, -CH_2), 6.35 (s, 1H, NH), 7.07 (d, 2H, Ar-H), 7.59-7.66 (m, 4H, Ar-H), 7.70 (s, 1H, Ar-H). MS (ESI) *m/z*: 313 (Expected mass: 311). Anal. Calcd. for $C_{17}H_{17}N_3O_3$ (in %): C, 65.88; H, 5.50; N, 13.50. Found: C, 60.50; H, 4.64; N, 13.55.

*4.4.5. N-((5-(2-Chloro-6-fluorophenyl)-1,3,4-oxadiazole-2-yl)methyl)-4-methoxyaniline **(4e)***

The compound **4e** was obtained by the reaction of **3e** (1.0 gm, 0.003 mmol) and iodobenzene diacetate (1.15 gm, 0.0035 mmol) using the general procedure described for **4(a-j)**. Pale brown solid. FT-IR (KBr, cm^{-1}): 3072 (aromatic C-H), 2961 (C-H of CH_2), 1660 (C=N), 1486 (C=C), 1380 (C-N), 1236 (C-O). ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.89 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.36 (s, 1H, NH), 7.06 (dd, 2H, Ar-H), 7.10-7.13 (t, 1H, Ar-H), 7.40-7.49 (m, 2H, Ar-H), 7.87 (dd, 2H, Ar-H), MS (ESI) *m/z*: 335 (Expected mass: 333). Anal. Calcd. for $C_{16}H_{13}ClFN_3O_2$ (in %): C, 57.58; H, 3.93; N, 12.59. Found: C, 57.65; H, 3.99; N, 12.50.

*4.4.6. N-((5-(2,4-Dichlorophenyl)-1,3,4-oxadiazole-2-yl)methyl)-4-methoxyaniline **(4f)***

The compound **4f** was obtained by the reaction of **3f** (1.0 gm, 0.0028 mmol) and iodobenzene diacetate (1.1 gm, 0.0031 mmol) using the general procedure described for **4(a-j)**. White solid. FT-IR (KBr, cm^{-1}): 3070 (aromatic C-H), 2944 (C-H of CH_2), 1629 (C=N), 1480 (C=C), 1386 (C-N), 1245 (C-O). ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.88 (s, 3H, -OCH_3), 4.60 (d, 2H, -CH_2), 6.35 (s, 1H, NH), 7.01 (dd, 2H, Ar-H), 7.76 (d, 2H, Ar-H), 7.88-7.94 (m, 3H, Ar-H). MS (ESI) *m/z*: 352 (Expected mass: 349). Anal. Calcd. for $C_{15}H_{10}Cl_2N_3O_3$ (in %): C, 53.75; H, 3.01; N, 12.54. Found: C, 53.58; H, 3.05; N, 12.34.

*4.4.7. N-((5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-yl)methyl)-4-methoxyaniline **(4g)***

The compound **4g** was obtained by the reaction of **3g** (1.0 gm, 0.003 mmol) and iodobenzene diacetate (1.18 gm, 0.0036 mmol) using the general procedure described for **4(a-j)**. Yellow solid. FT-IR (KBr, cm^{-1}): 3065 (aromatic C-H), 2950 (C-H of CH_2), 1634 (C=N), 1481 (C=C), 1380 (C-N), 1241 (C-O). ^1H NMR (DMSO-d_6, 400 MHz) δ: 3.87 (s, 3H, -OCH_3), 4.58 (d, 2H, -CH_2), 6.34 (s, 1H, NH), 7.10-7.18 (m, 4H, Ar-H), 7.76 (d, 2H, Ar-H), 8.10 (d, 2H, Ar-H). MS (ESI) *m/z*: 327 (Expected mass: 326). Anal. Calcd. for $C_{16}H_{14}N_4O_4$ (in %): C, 58.89; H, 4.32; N, 17.17. Found: C, 58.80; H, 4.30; N, 17.12.

*4.4.8. 4-Methoxy-N-((5-(4-propylphenyl)-1,3,4-oxadiazole-2-yl)methyl)aniline **(4h)***

The compound **4h** was obtained by the reaction of **3h** (1.0 gm, 0.003 mmol) and iodobenzene diacetate (1.15 gm, 0.0035 mmol) using the general procedure described for **4(a-j)**. White solid. FT-IR (KBr, cm^{-1}): 3075 (aromatic C-H), 2950 (C-H of CH_2), 1670 (C=N), 1462 (C=C), 1382 (C-N), 1229 (C-O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 0.92 (t, 3H, CH_3), 1.54-1.62 (m, 2H, CH_2), 2.49-2.51 (t, 2H, CH_2), 3.87 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.35 (s, 1H, NH), 7.10 (dd, 2H, Ar-H), 7.42 (dd, 2H, Ar-H), 7.81 (d, 2H, Ar-H), 7.89-7.92 (m, 2H, Ar-H). MS (ESI) *m/z*: 324 (Expected mass: 333). Anal. Calcd. for $C_{19}H_{21}N_3O_2$ (in %): C, 70.57; H, 6.55; N, 12.99. Found: C, 70.62; H, 6.52; N, 12.95.

4.4.9. 4-Methoxy-N-((5-(m-tolyl)-1,3,4-oxadiazole-2-yl)methyl)aniline ***(4i)***

The compound **4i** was obtained by the reaction of **3i** (1.0 gm, 0.0033 mmol) and iodobenzene diacetate (1.3 gm, 0.004 mmol) using the general procedure described for **4(a-j)**. White solid. FT-IR (KBr, cm^{-1}): 3063 (aromatic C-H), 2934 (C-H of CH_2), 1688 (C=N), 1469 (C=C), 1385 (C-N), 1231 (C-O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.22 (s, 3H, -CH_3), 3.88 (s, 3H, -OCH_3), 4.59 (d, 2H, -CH_2), 6.35 (s, 1H, NH), 7.17 (dd, 2H, Ar-H), 7.51 (s, 1H, Ar-H), 7.77-7.82 (m, 4H, Ar-H). MS (ESI) *m/z*: 297 (Expected mass: 295). Anal. Calcd. for $C_{17}H_{17}N_3O_2$ (in %): C, 69.14; H, 5.80; N, 14.23. Found: C, 69.10; H, 5.75; N, 14.18.

4.4.10. N-[{5-(4-Bromophenyl)-1,3,4-oxadiazole-2-yl}methyl]-4-methoxyaniline ***(4j)***

The compound **4j** was obtained by the reaction of **3j** (1.0 gm, 0.0027 mmol) and iodobenzene diacetate (1.34 gm, 0.004 mmol) using the general procedure described for **4(a-j)**. White solid. FT-IR (KBr, cm^{-1}): 3055 (aromatic C-H), 2921 (C-H of CH_2), 1682 (C=N), 1465 (C=C), 1380 (C-N), 1240 (C-O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.87 (s, 3H, -OCH_3), 4.60 (d, 2H, -CH_2), 6.34 (s, 1H, NH), 7.07 (d, 2H, Ar-H), 7.40 (d, 2H, Ar-H), 7.77 (d, 2H, Ar-H), 7.95 (d, 2H, Ar-H). MS (ESI) *m/z*: 362 (Expected mass: 360). Anal. Calcd. for $C_{16}H_{14}BrN_3O_2$ (in %): C, 53.55; H, 3.92; N, 11.67. Found: C, 53.23; H, 3.81; N, 11.7.

4.5. Antibacterial activity

Antibacterial activity of the newly synthesized compounds was determined in DMF by disc diffusion method on nutrient agar medium[18] by using Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Xanthomonas campestris* and *Escherichia coli*). In each Petri plate the sterile media (Nutrient Agar Medium, 15 mL) was uniformly smeared with cultures of both bacteria. Sterile discs of 10 mm diameter (Hi-Media) was placed in the Petri plates, to which 50 µL (1 mg/ml i.e., 50 µg/disc) of the new compounds were added. This included 50 µL of DMF as negative, bacteriomycin and gentamycin as positive control. The plates were incubated at 37 ± 2 ºC for 24 h and the zone of inhibition was determined.

4.6. Antifungal activity

The synthesized compounds were screened for the antifungal activity in DMF by poisoned food technique[19] against *Fusarium oxysporum*. Prepare Potato Dextrose Agar (PDA) media and about 15 mL of PDA was poured into each Petri plate and allowed to solidify. 5 mm disc of seven days old culture of the test fungi was placed at the center of the Petri plate and incubated at 26 °C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Nystatin was used as standard. All the synthesized compounds were tested (at the dosage of 500 µL of the new compounds/petriplate, where concentration was 0.1 mg/mL) by poisoned food technique.

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