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Novel pyrazoline amidoxime and their 1,2,4-oxadiazole analogues: Synthesis and pharmacological screening

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ABSTRACT

A novel series of pyrazoline amidoxime (**2a**–**d**) and pyrazoly-1,2,4-oxadiazole (**3a**–**p**) and (**4**) of pharmacological significance have been synthesised. Structures of newly synthesised compounds were characterized by spectral studies. New compounds were screened for their in vitro antioxidant, antimicrobial and antiinflammatory activities. Among the synthesized compounds, compound **2a**, **3l** and **3o** were found to be active antimicrobial agents in addition to having potent antioxidant activity, while the compound **3f** showed promising antiinflammatory activity in comparison with standard drug.

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Although pyrazoles are less common in nature, they became ubiquitous motif in numerous pharmaceutically active compounds. This is due to their wide spectrum of biological activity and easy preparation. In 1884 Knorr discovered the antipyretic action of a pyrazole derivative in man and he named the compound antipyrine. This stimulated interest in pyrazole chemistry. Pyrazole and its synthetic analogues have been found to exhibit antidiabetic,[1] antidepressant,[2] anticonvulsant,[3] antimicrobial,[4] analgesic,[5] and antitumour[6] activity and also serves as human acyl-CoA: cholesterol acyltransferase inhibitors.[7] In fact, Celecoxib, a pyrazole derivative is now widely used in the market as antiinflammatory drug.[8] The 1,2,4-oxadiazole, known as an ester isostere, is present in various biologically active compounds, such as benzodiazepine receptor ligands, muscarinic receptor agonists and 5-HT3 receptor antagonists.[9] 1,2,4-oxadiazole derivatives possess human tryptase inhibitory activity,[10] antitrypanosomal activity,[11] β-amyloid imaging agents in Alzheimer's disease,[12] genotoxic activity,[13] peptide inhibitory activity,[14] antihyperglycemic activity,[15] potential Combretastatin A-4 (CA-4) analogs[16] and oxadiazole mannich bases show antimyco-bacterial activity.[17]

Antioxidants play a vital role in the defense mechanism against oxidative damage induced by free radicals and ROS (Reactive Oxygen Species). Balanced oxidants (ROS) generation and detoxification in a normal cellular metabolism is important to keep the mammalian cells in a healthy condition. When a cell fails to detoxify the excessive ROS generated as a result of damaging species or low level of antioxidants they enter into a state of oxidative stress and is damaged.[18] High level of ROS can cause damage to cell structure, nucleic acids, membrane lipids and proteins.[19] They also damage purine and pyrimidine bases of DNA molecule, thus leading to mutation.[20] Oxidative stress on a cell due to high concentration of ROS can lead to a variety of disorders including cancer, neurodegenerative disorder, atherosclerosis and aging.[21] Many studies have suggested that antioxidants or other compounds that can neutralize free radicals may be of pivotal interest in the prevention of vascular diseases and some forms of cancer.[22]

The incidence of microbial infections has increased dramatically in recent years.[23] The wide spread use of antimicrobial drugs has resulted in the development of resistance to these drugs by pathogenic microorganisms and consequently, this has led to serious health hazards.[24] Though there are a number of antimicrobial drugs such as ampicillin, posaconazole etc., the preparation of these drugs involve multi steps and expensive chemicals. Thus, intense efforts in antimicrobial drug discovery are still needed to develop more promising, economical, and effective drugs for use in the clinical arena.[25]

Amidoxime is sometimes considered as bioisostere for carboxylic group, and there are some successful examples of drug

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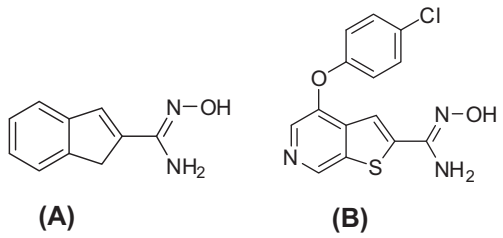
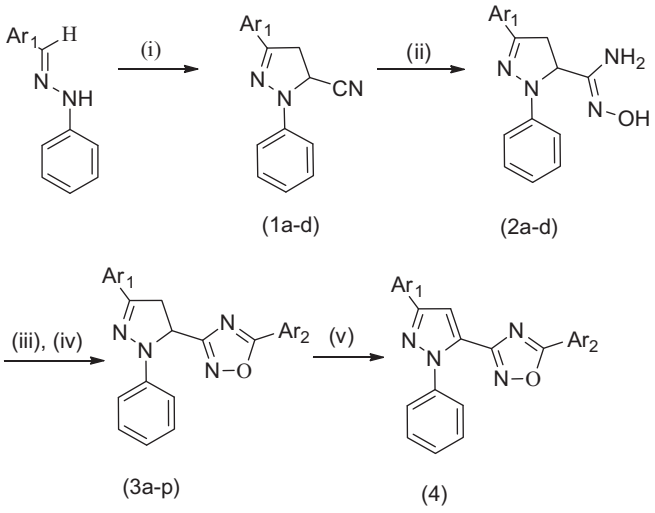


Figure 1. (A) Cardiotonic (B) abtiarthritic.



Scheme 1. Reagents and conditions: (i) acrylonitrile, chloramine-T, EtOH, reflux 3 h; (ii) $NH_2OH{\cdot}HCl$, Na_2CO_3, aq. EtOH, reflux 5–6 h; (iii) Ar^2-COOH, EDC·HCl, CH_2Cl, 0–30 °C 1–2 h, then rt for 6 h; (iv) heating 110 °C, 12 h; (v) Br_2, CH_2Cl_2, reflux 1 h.

candidates containing amidoxime moiety (Fig. 1).[26,27] A number of amidoximes have already been used or are currently being used in clinical trials as prodrugs for amidines, due to their ability to release NO.[28] Nevertheless, the main application of this building blocks subtype in drug design is related to the construction of 1,2,4-oxadiazole ring.

Pyrazoline/pyrazole incorporated with other biologically active heterocycles like oxazole, oxadiazole, triazole, thiazole, thiadiazole etc., was designed in an attempt to obtain synergistic chemotherapeutic activity with higher selectivity and less toxicity. Recently, pyrazole incorporated 1,2,4-triazoles and benzoxazoles,[29] pyrazole integrated with 1,3,4-oxadiazole,[30] 1-arylmethyl-3-aryl-*1H*-pyrazole linked with 1,2,4-oxadiazole were synthesized and observed in the enhancement of pharmacological effect.[31] Series of 1,2,4-oxadiazoles were prepared using amidoxime by acylation with acid chlorides, anhydrides or by reaction of amidoxime with carbonyl-containing compounds (ester, acids, aldehydes), or via interaction of amidoxime with acid amides, nitriles in presence of $ZnCl_2$, or in the course of cycloaddition of *N*-oxides to nitriles.[32–38]

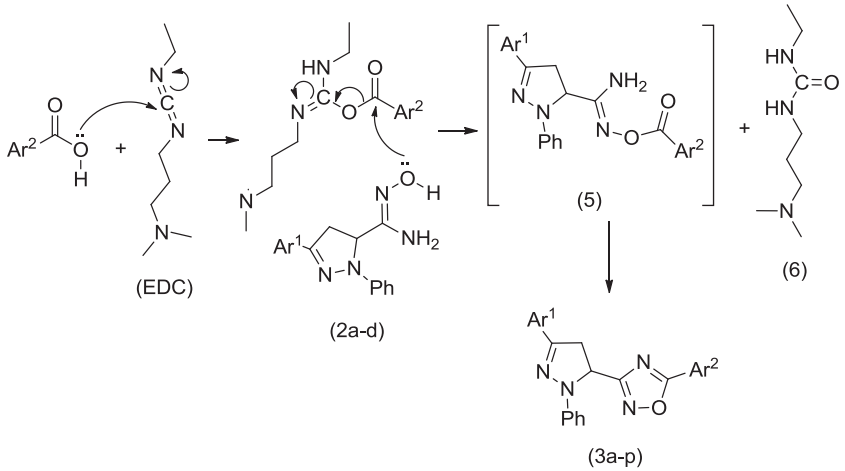
Prompted by these observations, we hereby report the synthesis of pyrazolyl-amidoximes and bis (heterocycle) bearing pyrazole and 1,2,4-oxadiazoles starting from inexpensive reagents in a simple procedure and evaluate them for in vitro antioxidant, antimicrobial and antiinflammatory activities.

The target compounds were prepared as shown in Scheme 1. (see Supplementary Informationfor detailed experimental procedure) The key intermediates 4,5-dihydro-1,3-diphenyl-*1H*-pyrazole-5-carbonitrile (**1a–d**) were synthesized utilizing a reported method.[39] The formation of pyrazolyl-1,2,4-oxadiazoles (**3a–p**) can be explained based on the plausible mechanism illustrated in (Scheme 2). Carboxyl group activation with EDC·HCl proceeds similarly to other carbodiimide couplings. An *O*-acylisourea intermediate is formed by the reaction of a carboxylic acid and the EDC·HCl. The *O*-acylisourea is a highly reactive species that readily reacts with amidoxime (**2a–d**) to obtain *N*-acyl-intermediate (**5**). EDC·HCl is transformed to the corresponding water soluble urea (**6**) during coupling reactions. Finally pyrazolyl-1,2,4-oxadiazoles (**3a–p**) was achieved by cyclodehydration of intermediate (**5**).

As reported in the literature the regioselectivity of the nitrile group in compound **1a** was further confirmed by the single crystal X-ray studies[40] of **2a** wherein amidoxime group is attached to the 5th carbon of the pyrazoline ring. The ORTEP diagram is as shown in Figure 2.

The structures assigned to the compounds were substantiated by their analytical and other spectral data. The structure of the compound (**2a**) was confirmed by single crystal X-ray studies. The IR spectra of all the synthesized compounds showed characteristic signals at 1640–1595 cm^{-1} for (C=N), 1570–1460 cm^{-1} for (C=C), 1260–1285 cm^{-1} for (C–N), and 1240–1230 cm^{-1} for (C–O). The absence of NH_2 stretch at 3248 cm^{-1} and appearance of C=N at 1620 cm^{-1}, also absence of NH_2 and OH peak at 5.49 and 9.3 ppm, respectively, in ^1H NMR confirms the formation of oxadiazole **3a**.

In the ^1H NMR spectra of compound **3a**, Ha, Hb and Hc protons of the pyrazoline ring were seen as a doublet of doublet. Among



Scheme 2. Proposed mechanism for pyrazoly-1,2,4-oxadiazole formation.

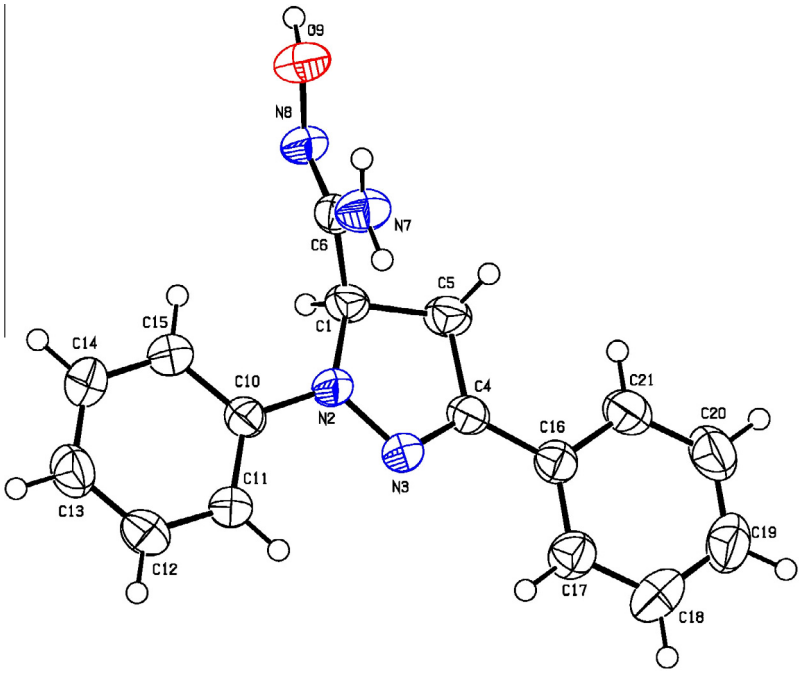


Figure 2. Perspective diagram of the molecule (**2a**) with 50% probability displacement ellipsoids.

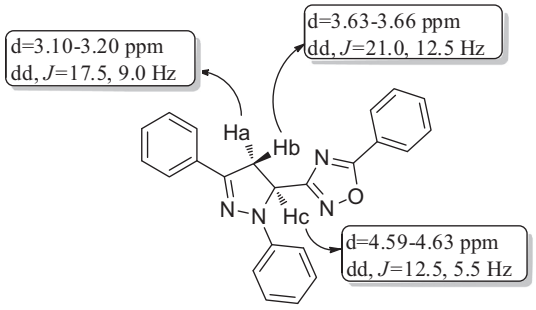


Figure 3. Proton chemical shifts and couplings of **3a**.

Ha, Hb and Hc protons, Hc is the most deshielded one due to its close proximity to nitrogen and oxadiazole moiety and it appeared as a doublet of doublets. The methylene protons of the pyrazoline ring (Ha and Hb), exhibited a typical ABX spin system with Hc as a doublet of doublets. (Fig. 3). In addition, the single crystal studies of **2a** confirmed that the connectivities of these protons to the carbons were also in accordance with the assigned structure.

The mass spectrum of all the compounds showed molecular ion peak at M+1 corresponding to its molecular formula, which confirmed its chemical structure. The IR, NMR, mass spectra and elemental analysis supported the structure of various synthesized bis-heterocycles (**3a**–**p**).

All the synthesized compounds **2**–**4** were evaluated for their in vitro antioxidant activity by various methods such as DPPH radical scavenging assay,[41] reducing power determination[42] and lipid peroxidation inhibitory activity[43] at three (10, 50, 100 μg/ml) different concentrations. The results of in vitro antioxidant activity are represented in Table 1. The investigation of antioxidant screening revealed that some of the tested compounds showed moderate to good antioxidant activity. The interaction of pyrazolyl-1,2,4-oxadiazole (**3a**–**p**) with stable DPPH free radical indicates their free radical scavenging ability. Of all the compounds in these series, **3f**, **3h**, **3o** and **3p** showed good interaction with the DPPH radical. The antioxidant activity of these compounds is related with their electron or hydrogen radical donating ability to DPPH radical, so that they become stable diamagnetic molecules. This might be the reason for the higher antioxidant activity. The maximum antioxidant activity of compounds was observed in the following order **3o** > **3h** ⩾ **3p** > **3f**. Particularly, the compound **3o** showed more promising DPPH RSA as compared to that of standard ascorbic acid. This could be due the presence of electron donating three-OCH_3 and one-OH group on benzene rings. The compounds **3c**, **3g** and **3k** have shown poor activity even if they process –OCH_3 group at *ortho* position. This indicates that the *ortho*-substituted benzene ring is less interactive with DPPH radical may be due to immensity of methoxy group and hence poor activity. It is interesting to notice that the compound **2d** with only one-OH group on benzene ring shows good interaction with DPPH radical and showed propitious DPPH RSA. The presence of free –NH_2 and –OH group in the amidoximes (**2a**–**d**) might add on to the electron donating capacity to DPPH radical thus increasing in activity. This is further supported by the fact that the compounds other than **3o**, **3h**, **3p**, and **3f**, showed less activity than compounds (**2a**–**d**), while, **3f** has shown more potent activity by reducing power determination. This may be due to two halogen atoms on benzene rings. All other compounds showed low to moderate reducing power activity. An intriguing lipid peroxidation inhibitory activity was observed for compound **2a** at 10 μg/mL concentration. This may be due to the ability of amidoximes to donate NO under mild oxidants.[44] NO can serve as a chain-terminating antioxidant and reduces oxidative injury to mammalian cells by attenuation of metal/peroxide oxidative chemistry, as well as lipid peroxidation.[45–47] It has also been proposed that, at higher level NO can enhance ROS toxicity due to its rapid reaction with superoxide (O_2^-) and CO_2 to form highly reactive oxidants peroxynitrite ($ONOO^-$) and nitrosoperoxocarbonate anion ($ONOOCO_2^-$) respectively. Thus NO can act as pro-oxidant at higher concentrations.[48] Prompted by these observations, we found out the effective concentration (EC) of compound **2a** required for lipid peroxidation inhibitory activity. From the Table 2, it is clear that compound **2a** showed (≈threefold) enhanced lipid peroxidation inhibitory activity than vitamin-E at 0.1 μg/mL concentration. The remaining compounds showed moderately good activity.

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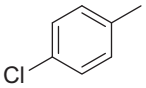
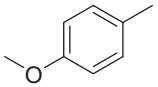
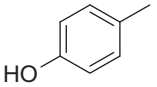
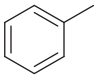
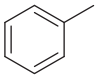
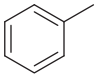
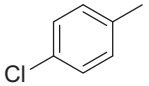
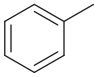
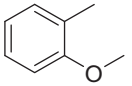
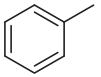
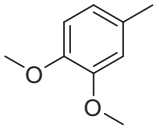
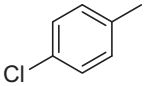
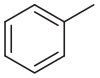
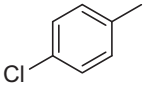
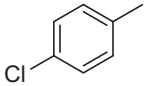
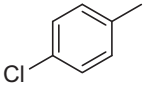
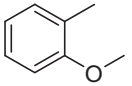
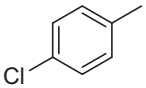
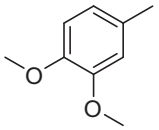
Table 1
Antioxidant activity of synthesized novel series of pyrazole–amideoxime (**2a–d**) and pyrazoly-1,2,4-oxadiazole (**3a–p**)

Compounds	Ar_1	Ar_2	% DPPH radical scavenging assay			% Reducing power determination			% Lipid peroxidation inhibitory activity		
			10 (μg/ml)	50	100	10 (μg/ml)	50	100	10 (μg/ml)	50	100
2a		–	16 ± 0.022	28 ± 0.091	51 ± 0.099	08 ± 0.091	12 ± 0.082	56 ± 0.088	32 ± 0.012	15 ± 0.019	14 ± 0.026
2b		–	29 ± 0.013	39 ± 0.032	69 ± 0.086	11 ± 0.083	28 ± 0.016	68 ± 0.023	18 ± 0.012	12 ± 0.080	14 ± 0.063
2c		–	18 ± 0.092	33 ± 0.013	55 ± 0.066	10 ± 0.019	22 ± 0.027	62 ± 0.082	10 ± 0.021	16 ± 0.011	14 ± 0.023
2d		–	24 ± 0.016	41 ± 0.086	76 ± 0.055	12 ± 0.036	34 ± 0.011	79 ± 0.045	12 ± 0.012	18 ± 0.020	20 ± 0.016
3a			18 ± 0.076	28 ± 0.036	39 ± 0.032	06 ± 0.019	16 ± 0.016	58 ± 0.061	21 ± 0.063	40 ± 0.042	57 ± 0.022
3b			23 ± 0.022	42 ± 0.012	67 ± 0.079	12 ± 0.086	22 ± 0.092	65 ± 0.012	34 ± 0.053	48 ± 0.238	73 ± 0.761
3c			14 ± 0.012	34 ± 0.082	44 ± 0.016	10 ± 0.016	22 ± 0.082	54 ± 0.082	11 ± 0.086	31 ± 0.130	60 ± 0.052
3d			26 ± 0.191	56 ± 0.111	66 ± 0.093	12 ± 0.019	29 ± 0.016	68 ± 0.061	14 ± 0.056	52 ± 0.026	71 ± 0.048
3e			23 ± 0.226	40 ± 0.183	68 ± 0.082	13 ± 0.086	26 ± 0.092	62 ± 0.012	31 ± 0.010	44 ± 0.058	71 ± 0.276
3f			29 ± 0.10	53 ± 0.099	82 ± 0.093	16 ± 0.016	32 ± 0.082	86 ± 0.082	34 ± 0.044	58 ± 0.080	83 ± 0.045
3g			24 ± 0.016	30 ± 0.117	64 ± 0.025	12 ± 0.019	23 ± 0.016	68 ± 0.061	21 ± 0.088	38 ± 0.097	67 ± 0.128
3h			30 ± 0.226	59 ± 0.168	86 ± 0.298	14 ± 0.086	29 ± 0.092	75 ± 0.012	30 ± 0.095	52 ± 0.058	73 ± 0.446

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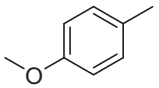
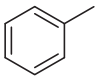
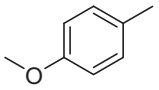
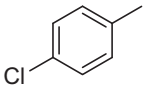
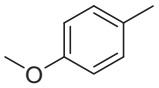
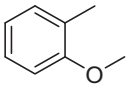
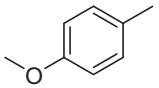
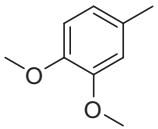
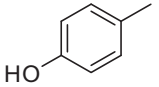
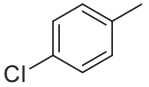
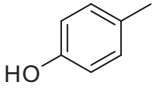
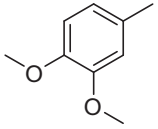
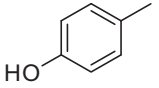
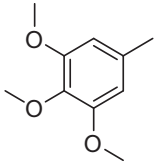
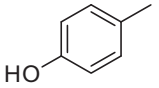
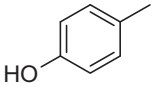
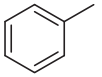
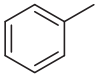
3i			19 ± 0.078	36 ± 0.289	51 ± 0.247	11 ± 0.016	20 ± 0.082	64 ± 0.082	26 ± 0.456	40 ± 0.025	62 ± 0.085
3j			22 ± 0.181	52 ± 0.008	72 ± 0.410	12 ± 0.019	23 ± 0.016	58 ± 0.061	34 ± 0.014	48 ± 0.133	70 ± 0.011
3k			20 ± 0.036	41 ± 0.049	53 ± 0.056	11 ± 0.086	24 ± 0.092	65 ± 0.012	21 ± 0.051	33 ± 0.121	57 ± 0.206
3l			29 ± 0.151	48 ± 0.096	67 ± 0.075	13 ± 0.016	23 ± 0.082	74 ± 0.082	34 ± 0.013	49 ± 0.018	76 ± 0.024
3m			30 ± 0.191	51 ± 0.080	70 ± 0.021	14 ± 0.019	26 ± 0.016	76 ± 0.061	40 ± 0.885	61 ± 0.025	80 ± 0.066
3n			31 ± 0.055	53 ± 0.041	76 ± 0.086	18 ± 0.086	24 ± 0.092	75 ± 0.012	32 ± 0.113	45 ± 0.044	73 ± 0.029
3o			34 ± 0.065	63 ± 0.034	91 ± 0.091	16 ± 0.016	26 ± 0.082	76 ± 0.082	42 ± 0.091	62 ± 0.032	87 ± 0.078
3p			31 ± 0.035	47 ± 0.155	86 ± 0.229	12 ± 0.011	30 ± 0.016	76 ± 0.066	34 ± 0.013	58 ± 0.038	78 ± 0.016
4			18 ± 0.076	28 ± 0.036	39 ± 0.032	10 ± 0.086	14 ± 0.092	65 ± 0.012	26 ± 0.296	40 ± 0.114	67 ± 0.178
Ascorbic acid	–	–	32 ± 0.018	57 ± 0.083	94 ± 0.012	14 ± 0.183	32 ± 0.112	82 ± 0.163	ND	ND	ND
Vitamin-E	–	–	ND	ND	ND	ND	ND	ND	47 ± 0.203	62 ± 0.188	92 ± 0.110

Values are expressed as mean ± standard deviation ($n = 3$).

Table 2
Effective concentration (EC) of **2a** for lipid peroxidation inhibitory activity

Compound	Conc. in μg/ml								
	0.01	0.05	0.1	0.25	0.5	0.75	1.0	2.5	5.0
2a	0.7 ± 0.203	1.3 ± 0.203	8.4 ± 0.203	8.0 ± 0.203	8.2 ± 0.203	8.5± 0.203	9.5 ± 0.203	14.7 ± 0.203	21.0 ± 0.203
Vitamine-E	1.2 ± 0.203	2.0 ± 0.203	3.4 ± 0.203	4.1 ± 0.203	6.0 ± 0.203	7.7 ± 0.203	9.3 ± 0.203	17.0 ± 0.203	26.0 ± 0.203

Table 3
'Antimicrobial activity of pyrazole–amideoxime (**2a**–**d**) and pyrazolyl-1,2,4-oxadiazole (**3a**–**p**) based on the measurement of the zone of the inhibition grow'

Compounds	Antibacterial activity										Antifungal activity			
	Gram positive				Gram negative									
	B. cereus		*S. aureus*		*E. coli*		*K. pneumonia*		*S. flexneri*		*A. flavus*		*A. niger*	
	50 μg/ml ± SD	100 μg/ml ± SD	50 μg/ml ± SD	100 μg/ml ± SD	50 μg/ml ± SD	100 μg/ml ± SD	50 μg/ml ± SD	100 μg/ml ± SD	50 μg/ml ± SD	100 μg/ml ± SD	50 μg/ml ± SD	100 μg/ml ± SD	50 μg/ml ± SD	100 μg/ml ± SD
2a	02 ± 0.16	03 ± 0.10	03 ± 0.23	06 ± 0.11	02 ± 0.13	07 ± 0.10	02 ± 0.09	08 ± 0.23	03 ± 0.22	10 ± 0.10	04 ± 0.19	04 ± 0.22	02 ± 0.20	03 ± 0.16
2b	04 ± 0.18	08 ± 0.15	03 ± 0.50	05 ± 0.10	03 ± 0.08	10 ± 0.08	08 ± 0.11	10 ± 0.25	08 ± 0.13	12 ± 0.11	06 ± 0.14	10 ± 0.50	08 ± 0.09	08 ± 0.12
2c	04 ± 0.12	16 ± 0.13	02 ± 0.18	12 ± 0.02	03 ± 0.11	08 ± 0.11	06 ± 0.21	09 ± 0.24	06 ± 0.15	10 ± 0.15	05 ± 0.11	10 ± 0.10	10 ± 0.12	12 ± 0.22
2d	03 ± 0.10	03 ± 0.06	02 ± 0.24	08 ± 0.53	10 ± 0.05	16 ± 0.07	08 ± 0.11	12 ± 0.11	12 ± 0.08	20 ± 0.14	05 ± 0.06	12 ± 0.42	10 ± 0.22	14 ± 0.52
3a	04 ± 0.13	08 ± 0.27	02 ± 0.13	08 ± 0.16	03 ± 0.15	08 ± 0.11	04 ± 0.22	06 ± 0.08	04 ± 0.13	08 ± 0.12	09 ± 0.05	12 ± 0.55	10 ± 0.35	14 ± 0.11
3b	06 ± 0.11	10 ± 0.11	08 ± 0.16	10 ± 0.17	04 ± 0.17	18 ± 0.23	05 ± 0.10	08 ± 0.13	06 ± 0.12	10 ± 0.22	12 ± 0.08	14 ± 0.48	11 ± 0.28	20 ± 0.18
3c	02 ± 0.09	08 ± 0.25	02 ± 0.17	06 ± 0.24	04 ± 0.25	06 ± 0.22	03 ± 0.12	06 ± 0.19	03 ± 0.12	08 ± 0.11	10 ± 0.18	12 ± 0.69	08 ± 0.44	14 ± 0.20
3d	08 ± 0.12	14 ± 0.21	08 ± 0.13	12 ± 0.16	08 ± 0.23	12 ± 0.09	08 ± 0.52	14 ± 0.18	07 ± 0.20	10 ± 0.18	10 ± 0.14	14 ± 0.19	12 ± 0.46	18 ± 0.14
3e	05 ± 0.05	08 ± 0.11	03 ± 0.22	11 ± 0.17	04 ± 0.17	10 ± 0.11	06 ± 0.15	08 ± 0.11	06 ± 0.11	08 ± 0.16	12 ± 0.16	18 ± 0.11	09 ± 0.08	18 ± 0.08
3f	06 ± 0.09	12 ± 0.16	06 ± 0.04	12 ± 0.19	08 ± 0.15	10 ± 0.18	06 ± 0.16	10 ± 0.12	08 ± 0.19	12 ± 0.12	15 ± 0.12	25 ± 0.19	20 ± 0.15	26 ± 0.10
3g	04 ± 0.11	08 ± 0.13	05 ± 0.42	10 ± 0.10	04 ± 0.11	08 ± 0.14	06 ± 0.10	08 ± 0.21	06 ± 0.23	08 ± 0.20	09 ± 0.11	10 ± 0.20	10 ± 0.22	20 ± 0.11
3h	08 ± 0.12	14 ± 0.19	06 ± 0.36	12 ± 0.20	08 ± 0.30	12 ± 0.13	09 ± 0.11	11 ± 0.26	10 ± 0.22	16 ± 0.19	14 ± 0.15	21 ± 0.18	14 ± 0.13	27 ± 0.16
3i	03 ± 0.18	12 ± 0.17	02 ± 0.22	12 ± 0.11	06 ± 0.18	08 ± 0.08	06 ± 0.12	08 ± 0.22	07 ± 0.09	08 ± 0.14	07 ± 0.13	10 ± 0.16	10 ± 0.41	18 ± 0.20
3j	04 ± 0.15	13 ± 0.22	05 ± 0.19	12 ± 0.14	08 ± 0.22	10 ± 0.19	07 ± 0.25	10 ± 0.18	08 ± 0.11	08 ± 0.18	12 ± 0.11	18 ± 0.10	12 ± 0.36	20 ± 0.12
3k	04 ± 0.06	08 ± 0.21	04 ± 0.35	08 ± 0.10	06 ± 0.20	08 ± 0.11	07 ± 0.33	08 ± 0.11	06 ± 0.12	10 ± 0.21	10 ± 0.14	12 ± 0.08	12 ± 0.21	22 ± 0.23
3l	12 ± 0.08	16 ± 0.14	10 ± 0.22	18 ± 0.16	08 ± 0.15	16 ± 0.17	08 ± 0.40	18 ± 0.16	10 ± 0.20	24 ± 0.14	16 ± 0.13	20 ± 0.16	16 ± 0.11	30 ± 0.16
3m	08 ± 0.13	10 ± 0.06	06 ± 0.45	10 ± 0.14	08 ± 0.11	14 ± 0.12	10 ± 0.17	12 ± 0.21	08 ± 0.32	18 ± 0.23	10 ± 0.21	18 ± 0.11	12 ± 0.41	18 ± 0.31
3n	10 ± 0.11	16 ± 0.33	08 ± 0.04	18 ± 0.20	07 ± 0.18	12 ± 0.14	08 ± 0.19	12 ± 0.18	10 ± 0.22	18 ± 0.19	14 ± 0.52	20 ± 0.52	18 ± 0.60	26 ± 0.23
3o	12 ± 0.02	18 ± 0.07	10 ± 0.11	18 ± 0.06	10 ± 0.31	16 ± 0.04	12 ± 0.02	18 ± 0.23	14 ± 0.08	22 ± 0.07	14 ± 0.11	26 ± 0.41	18 ± 0.33	30 ± 0.10
3p	06 ± 0.06	10 ± 0.13	08 ± 0.15	12 ± 0.19	12 ± 0.28	20 ± 0.11	10 ± 0.16	16 ± 0.17	14 ± 0.14	22 ± 0.11	12 ± 0.19	20 ± 0.25	14 ± 0.14	22 ± 0.21
4	05 ± 0.11	08 ± 0.22	03 ± 0.41	06 ± 0.13	04 ± 0.52	06 ± 0.21	03 ± 0.12	06 ± 0.08	03 ± 0.18	09 ± 0.23	10 ± 0.15	10 ± 0.41	09 ± 0.23	18 ± 0.08
Chloramphenicol	10 ± 0.07	18 ± 0.11	09 ± 0.11	16 ± 0.14	10 ± 0.04	18 ± 0.06	12 ± 0.18	20 ± 0.09	10 ± 0.18	24 ± 0.21	–	–	–	–
Fluconazole	–	–	–	–	–	–	–	–	–	–	16 ± 0.18	28 ± 0.08	20 ± 0.36	30 ± 0.11

[a]Zone of inhibition (Mean six replicate ± standard deviation).

The synthesized compounds were evaluated for in vitro antimicrobial activity against *Bacillus cereus* (MTCC 8372), *Staphylococcus aureus* (MTCC 96), (gram-positive bacteria), *Escherichia coli* (MTCC 724), *Klebsiella pneumonia* (MTCC 3384), *Shigella flexneri* (MTCC 1457), (gram-negative bacteria) and two fungi *Aspergillus flavus* (MTCC 873), *Aspergillus niger* (MTCC 281) by disc diffusion method.[49] The results are summarized in Tables 3 and 4. The results revealed that, compounds **3l** and **3o** showed excellent antimicrobial activity against all the tested strains of microbes, while compounds **2d**, **3d**, **3h** and **3p** exhibited good to potent antimicrobial activity. Of the five tested bacterial strains, gram-positive bacteria were inhibited mostly by compounds **2c**, **3d**, **3i**, **3j** and **3k** at a concentration of 100 μg/mL was due to the presence of electron donating $-OCH_3$ group. While the gram-negative bacteria were inhibited by compounds **2d**, **3n**, **3m** and **3p** this may be due to the presence of –OH group. This was further confirmed by the fact that the compounds containing both –OH and $-OCH_3$ groups were active against both types of bacteria. The activity is considerably affected by substituents present at the *para* position of phenyl ring. The compounds containing $-OCH_3$ group on *ortho* position (**3c**, **3g**, **3k**,) were less active against both bacterial and fungal strains. While the compounds containing electron withdrawing –Cl group were less active against bacterial strains but they possess good antifungal activity.

The newly synthesized compounds **2**–**4** were tested for *in vitro* antiinflammatory activity.[50–52] Compared to the standard, they have shown acceptable antiinflammatory activity. In vitro antiinflammatory activity of compounds is summarized in Table 5. The results revealed that the compounds, **3f**, **3m**, **3p** and **2d** exhibited moderate antiinflammatory activities. Among all the tested compounds **3f** was found to be more potent. The compounds **2a**, **3e**, **3o**, **2b** and **3n**, showed good activity, while others showed weak to moderate activities.

In summary, a series of novel pyrazoline amidoximes (**2a**–**d**) and their 1,2,4-oxadiazole analogues (**3a**–**p**) and **4** was prepared with moderate to good yields. The entire series of compounds were characterized by IR, NMR and mass spectral data. All the synthesized compounds **2**–**4** have been investigated for their in vitro antioxidant, antimicrobial and antiinflammatory activity. Among the synthesized compounds, compound **2a** showed superior antilipid peroxidation activity, **3o** showed promising DPPH radical scavenging activity, while the compounds **3l** and **3o** showed excellent antimicrobial activity, compound **3f** showed potent antiinflammatory activities in comparison with standard drug. Accordingly, these novel classes of pyrazoline amidoximes and their 1,2,4-oxadiazole analogues presented in our laboratory emerged as a valuable lead series that might be useful as antioxidant, antibacterial, antifungal and antiinflammatory agents and hence promising candidates for

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Table 4
Antimicrobial activity (MIC) of compounds pyrazole–amideoxime (**2a–d**) and pyrazoly-1,2,4-oxadiazole (**3a–p**)

Compounds	Antibacterial activity					Antifungal activity	
	Gram positive		Gram negative				
	B. cereus	*S. aureus*	*E. coli*	*K. pneumonia*	*S. flexneri*	*A. flavus*	*A. niger*
	μg/ml	μg/ml	μg/ml	μg/ml	μg/ml	μg/ml	μg/ml
2a	50	40	50	40	40	30	50
2b	30	40	40	20	20	25	20
2c	35	50	55	25	25	30	15
2d	40	50	15	20	10	35	15
3a	30	50	50	30	20	15	15
3b	25	20	30	30	25	10	10
3c	55	55	35	55	50	15	20
3d	20	20	20	20	25	10	10
3e	30	50	45	25	25	10	15
3f	25	20	20	25	20	10	5
3g	35	30	35	25	25	15	15
3h	20	25	20	15	15	10	10
3i	45	55	25	25	25	25	10
3j	40	30	25	25	25	10	10
3k	35	35	25	25	25	15	10
3l	10	15	20	20	15	10	10
3m	20	25	20	15	20	15	10
3n	15	20	25	25	15	10	5
3o	10	15	15	10	10	10	5
3p	25	20	10	15	10	10	10
4	35	55	40	50	55	15	20
Chloramphenicol	10	10	10	10	10	—	—
Fluconazole	—	—	—	—	—	5	5

[a](Mean six replicate ± standard deviation).

Table 5
In vitro antiinflammatory activity of compounds (**2a–d**) and (**3a–p**)

Compounds	Mean absorbance ± SD	% Inhibition of denaturation
2a	0.2456 ± 0.018	61.70
2b	0.3210 ± 0.007	59.06
2c	0.2498 ± 0.020	23.78
2d	0.3501 ± 0.026	63.48
3a	0.2489 ± 0.023	23.33
3b	0.3102 ± 0.010	53.71
3c	0.2501 ± 0.011	23.93
3d	0.2566 ± 0.052	27.15
3e	0.3228 ± 0.010	59.96
3f	0.3600 ± 0.041	78.39
3g	0.3165 ± 0.022	56.83
3h	0.3098 ± 0.026	53.51
3i	0.2488 ± 0.010	23.29
3j	0.3178 ± 0.020	57.48
3k	0.2566 ± 0.031	27.15
3l	0.2622 ± 0.019	29.93
3m	0.3523 ± 0.008	74.58
3n	0.3198 ± 0.018	58.47
3o	0.3215 ± 0.026	59.31
3p	0.3365 ± 0.022	69.74
4	0.2764 ± 0.010	36.96
Diclofenac sodium	0.3721 ± 0.013	84.39
Control	0.2018 ± ± 0.018	—

SD = standard deviation (average of three determination).

further efficacy evaluation. Further, detailed studies are required to understand the mechanism of action of these compounds.

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Supplementary data

Supplementary data (experimental details, IR, NMR, MS and elementary analysis) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.06.042.

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