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Novel semicarbazones based 2,5-disubstituted-1,3,4-oxadiazoles: One more step towards establishing four binding site pharmacophoric model hypothesis for anticonvulsant activity

Harish Rajak [a,*], Ravitas Deshmukh [a], Ravichandran Veerasamy [b], Ajay Kumar Sharma [c], Pradeep Mishra [d], Murli Dhar Kharya [e]

[a] Medicinal Chemistry Research Laboratory, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur 495 009, CG, India
[b] Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia
[c] Pharmacology Research Laboratory, Department of Pharmacy, G.S.V.M., Medical College, Kanpur 208 002, UP, India
[d] GLA Institute of Pharmaceutical Sciences and Research, Mathura 281 406, UP, India
[e] Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar 470 003, MP, India

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ABSTRACT

A series of novel N^1-{5-[(naphthalene-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl}-N^4-(4-substitutedbenzaldehyde)-semicarbazone, N^1-{5-[(naphthalene-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl}-N^4-[1-(4-substitutedphenyl)ethanone]-semicarbazone and N^1-{5-[(naphthalene-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl}-N^4-[1-(4-substitutedphenyl) (phenyl) methanone]-semicarbazone were designed and synthesized on the basis of semicarbazone based pharmacophoric model to meet the structural requirements necessary for anticonvulsant activity. The anticonvulsant activities of the compounds were investigated using maximal electroshock seizure (MES), subcutaneous pentylenetrtrazole (*sc*PTZ) and subcutaneous strychnine (*sc*STY) models. Some of the selected active compounds were subjected to GABA assay to confirm their mode of action. The efforts were also made to establish structure activity relationships among synthesized compounds. The results of the present studying validated that the pharmacophoric model with four binding sites is essential for anticonvulsant activity.

Epilepsy is a ubiquitous neurological disorder, characterized by paroxysmal cerebral dysrhythmia, manifested as brief episodes (seizures) of loss or disturbance of consciousness, often followed by convulsions. About 50 million people worldwide have epilepsy, with almost 90% of these people being in developing countries.[1] Epilepsy also affects about 4% of individuals over their lifetime. The incidence of epilepsy is highest among children below 7 years-of-age and in individuals of above 55 years. Epilepsy is the third most widely spread neurological disorder found in the elderly after cerebrovascular disease and dementia. Despite the development of several new anticonvulsants, over 30% of people with epilepsy do not have seizure control and others do so only at the expense of significant dose-related toxicity and peculiar adverse effects that range in harshness from minimal brain impairment and megaloblastic anemia to death from aplastic anemia or hepatic failure.[2,3] These limitations with conventional antiepileptic drugs demand the need for the development of a more effective and safer antiepileptic drug.

Several investigations have recognized aryl semicarbazones as a structurally novel class of compounds with noteworthy anticonvulsant activity.[4–6] On the other hand, several investigations have also revealed the anticonvulsant potential of 1,3,4-oxadiazole analogues.[7–9]

A pharmacophoric model has been put forward for antiepileptic activity owing to conformational investigations on prevailing anticonvulsant drugs such as phenytoin, carbamazepine, rufinamide, lamotrigine and phenobarbitone.[10,11] This semicarbazones based pharmacophoric model comprises of following four essential binding sites: (i) An aryl hydrophobic binding site (A) with halo substituent preferably at para position; (ii) A hydrogen bonding domain (HBD); (iii) An electron donor group (D) and (iv) Another hydrophobic-hydrophilic site regulating the pharmacokinetic properties of the anticonvulsant (C) (Fig. 1). In earlier studies on this pharmacophoric model, our research group has established that the presence of halogen substituted aryl group near the semicarbazone moiety is one of the crucial parameters for anticonvulsant activity.[12,13]

The title compounds were prepared using the synthetic strategy described in Scheme 1. The ethyl (naphthalene-2-yloxy) acetate **II** was synthesized by esterification of 2-naphthol **I** with ethylbromoacetate in the presence of anhydrous potassium carbonate using anhydrous acetone as solvent. Compound **II,** on

* Corresponding author. Tel.: +91 9827911824; fax: +91 7752 417720.
E-mail address: harishdops@yahoo.co.in (H. Rajak).

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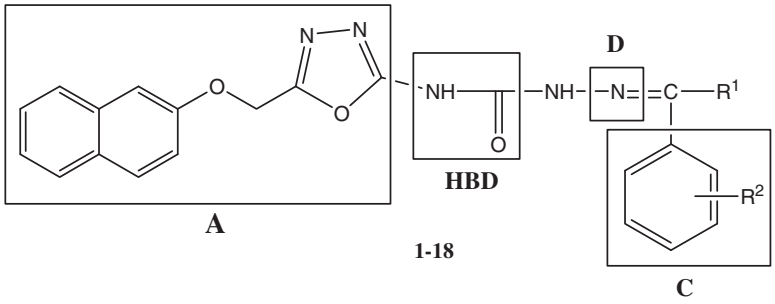


Figure 1. Semicarbazone based pharmacophoric model and its vital structural features present in title compounds: (A) hydrophobic aryl ring system, (HBD) hydrogen binding domain, (D) electron donor moiety and (C) distal aryl ring.

reaction with hydrazine hydrate in absolute ethanol, resulted in the formation of 2-(naphthalene-2-yloxy) acetohydrazide **III**.[14] The 2-amino-5-(2-naphthyloxymethyl)-1,3,4-oxadiazole **IV** was synthesized by the reaction of hydrazide **III** with cyanogen bromide in the presence of Na_2CO_3. The compound **IV** on reaction with phenyl formate in the presence of chloroform resulted in the formation of phenyl{5-[(naphthalen-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl}carbamate **V**. *N*-{5-[(Naphthalen-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl}hydrazin-ecarboxamide **VI** was prepared by condensation of carbamate **V** with hydrazine hydrate in the presence of methylene dichloride. The title compounds **1**–**18** were synthesized by reaction of hydrazinecarboxamide **VI** with appropriate aldehyde and ketone.[15]

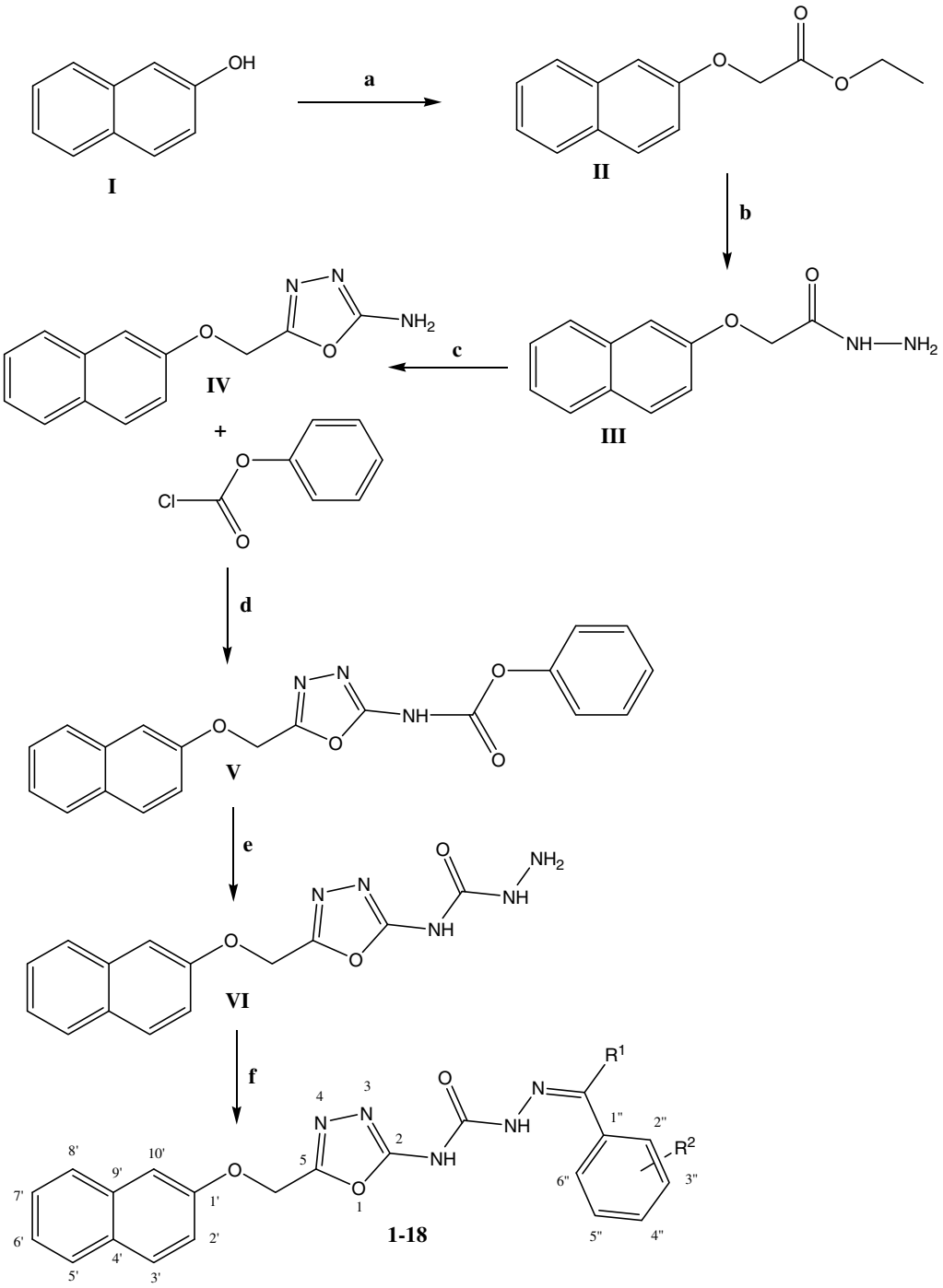
The anticonvulsant screening[16–18] was accomplished using male albino mice (swiss, 18–25 g) and rat (Wistar 100–150 g). The anticonvulsant potential of the test compounds was assessed by three models namely, maximal electroshock seizure (MES), subcutaneous pentylenetrtrazole (*sc*PTZ), and subcutaneous strychnine (*sc*STY) models. Acute neurological toxicity in mice was evaluated by rotorod test.[19] All the synthesized oxadizole analogues were screened for their anticonvulsant potential through MES and *sc*PTZ models in doses of 30, 100, 300 mg/kg by intraperitoneal (ip) injection. The majority of the compounds that is, **1**, **4**–**7**, **9**–**11** and **13** to **18** exhibited activity in either of the MES, *sc*PTZ or *sc*STY models after 4 h, indicating that the test compounds are slow acting anticonvulsants. The data indicates that 72% of the compounds that is, **1**, **3**, **4**, **6**, **7**, **9**, **11**, **13**–**18** were active in the MES screening, 44% of the compounds that is, **5**, **7**, **10**, **11**, **12**, **14**, **16** and **17** were active in the *sc*PTZ test and 39% of the compounds that is, **3**, **6**, **8**, **11**, **14**, **16** and **17** were active in *sc*STY test. These results show that compounds possess some MES selectivity (Tables 1 and 2). The subcutaneous strychnine (*sc*STY) test provides some hints about possible interaction of test compounds with glycine receptors. The activity of compounds that is, **3**, **6**, **8**, **11**, **14**, **16** and **17** in *sc*STY test indicates that their anticonvulsant activity may be through inhibitory glycine receptors.

On correlating the structures of the synthesized compounds with their biological activity, it has been observed that compounds bearing the groups like nitro, hydroxy on distant phenyl ring possess high potency in MES, *sc*PTZ, and *sc*STY tests. On the other hand, replacement of these groups with methoxy or methyl groups on the distant phenyl ring has resulted in compounds with lesser anticonvulsant activity. Replacement of the proton on the carbimino carbon atom by methyl group that is, **10**–**14** or phenyl ring that is, **15**–**18** has exhibited alteration in biological activity due to increase in the dimension of the group at this position of the molecule. Compounds with phenyl ring exhibited considerable anticonvulsant activity in comparison to methyl group. The increase in anticonvulsant activity of test compounds **15**–**18** may be attributed to the existence of phenyl substitution which might be accountable for additional van der Waals bonding to the binding site.

4-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) and is largely concerned with epilepsy.[20,21] It has been observed that inhibition of GABAergic neurotransmission or administration of GABA antagonists has been shown to advance and accelerate seizures,[22] while enhancement of GABAergic neurotransmission has proved effective as anticonvulsants in a variety of experimental models of epilepsy and in epileptic patients.[23] The aryl semicarbazones have been found to possess anticonvulsant activity through GABA mediation.[24,25] Most active compounds found after initial anticonvulsant screening were subjected to neurochemical estimation of GABA level in adult Wistar rat brain. The determination of GABA in brain extract is based on the use of an enzyme system found in the bacterium *Pseudamonas fluorescens* grown on pyrrolidine which converts GABA to succinate via transamination and oxidation coupled to triphosphopyrindine nucleotide reduction. The animals were sacrificed after 2 h of drug administration by decapitation and the brain regions that is, midbrain, medulla oblongata and cerebellum were dropped into separate vessel containing 6–8 ml of ice-cold 80% ethanol and processed further as per reported procedure.[26,27] Some of these compounds were found to increase the GABA level many times as compared to the control which confirmed that the presently studied aryl semicarbazones containing 1,3,4-oxadiazole moiety exhibit anticonvulsant activity via GABA mediation (Table 3).

We have designed and synthesized the title compounds while remembering the fact that a number of clinically active anticonvulsants possess a nitrogen hetero atomic system with one or two phenyl rings and at least one carbonyl group in their structure. The structure of the title compounds **1**–**18** satisfied all the pharmacophoric structural requirements that is, presence of 5-[(naphthalene-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl moiety as hydrophobic portion, N as electron donor system, and another hydrophobic distal aryl ring responsible for controlling the pharmacokinetic properties of the anticonvulsant. Thus, these findings confirmed the long-established four binding site hypothesis for semicarbazones. In the present study N^1-{5-[(naphthalene-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl}-N^4-[1-(4-nitrophenyl) (phenyl) methanone]-semicarbazone **17** emerged out as the most active compound with a wide spectrum of anticonvulsant activity without any neurotoxicity.

In conclusion, a series of novel N^1-{5-[(naphthalene-2-yloxy)-methyl]-1,3,4-oxadiazol-2-yl}-N^4-(4-substitutedbenzaldehyde)-semicarbazone **1**–**9**, N^1-{5-[(naphthalene-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl}-N^4-[1-(4-substitutedphenyl)ethanone]-semicarbazone **10**–**14,** and N^1-{5-[(naphthalene-2-yloxy)methyl]-1,3,4-oxadiazol-2-yl}-N^4-[1-(4-substitutedphenyl) (phenyl) methanone]-semicarbazone **15**–**18** were synthesized to meet structural requirements necessary for anticonvulsant activity. The result of the GABA assay implies that the test compounds have inhibited or attenuated seizures by facilitating GABAergic neurotransmission. Our results validated that the pharmacophoric model with four binding sites



Compound Code	R^1	R^2	Compound Code	R^1	R^2	Compound Code	R^1	R^2
1	H	H	7	H	3-OH	13	CH_3	4-OH
2	H	4-Cl	8	H	3-OCH_3 4-OH	14	CH_3	4-NH_2
3	H	4-NO_2	9	H	4-$N(CH_3)_2$	15	C_6H_5	H
4	H	4-CH_3	10	CH_3	4-Cl	16	C_6H_5	4-OH
5	H	4-OCH_3	11	CH_3	4-NO_2	17	C_6H_5	4-NO_2
6	H	4-OH	12	CH_3	4-CH_3	18	C_6H_5	4-OCH_3

Scheme 1. Synthesis of 2,5-disubstituted-1,3,4-oxadiazole analogues **1–18**. Reaction conditions: (a) anhydrous K_2CO_3, anhydrous acetone, reflux for 16 h, 60%; (b) $NH_2NH_2{\cdot}H_2O$, absolute ethanol, reflux for 6 h, 64%; (c) BrCN, $NaHCO_3$, ethanol, 55–60 °C, 1.5 h, 62%; (d) $CHCl_3$, $N(C_2H_5)$, 56%; (e) $NH_2NH_2{\cdot}H_2O$, CH_2Cl_2, 59%; (f) aldehyde or ketone, glacial acetic acid, ethanol, 53–69%.

Table 1
Anticonvulsant and neurotoxicity screening of 2,5-disubstituted 1,3,4-oxadiazoles

Compound[a]	MES screening[b]		*sc*PTZ screening[b]		*sc*STY screening[c]		NT screening[b]	
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
1	—	300	—	—	—	—	—	300
2	—	—	—	—	—	—	300	—
3	300	—	—	—	—	300	—	300
4	—	300	—	—	—	—	—	—
5	—	—	—	300	—	—	—	—
6	100	300	—	—	100	300	100	100
7	—	300	—	300	—	—	—	300
8	—	—	—	—	—	300	300	—
9	100	300	—	—	—	—	—	300
10	—	—	—	300	—	—	—	—
11	100	300	—	300	—	300	—	—
12	—	—	300	—	—	—	300	—
13	100	300	—	—	—	—	—	300
14	—	300	—	300	—	300	—	—
15	—	300	—	—	—	—	300	—
16	100	300	—	300	—	300	—	—
17	100	300	100	300	—	300	—	—
18	—	300	—	—	—	—	—	300
Phenytoin	30	30	—	—	—	100	100	100
Carbamazepine	30	100	100	300	—	—	100	300
Na valproate	300	—	300	—	300	—	—	—
Ethosuximide	—	—	100	300	300	—	—	—

Abbreviations: MES = maximal electroshock seizure; *sc*PTZ = subcutaneous pentylenetetrazole; *sc*STY = subcutaneous strychnine; NT = neurotoxicity.
[a] 30, 100 and 300 mg/kg of doses were administered ip in the animals. The data in the table indicates the minimal dose whereby biological activity was demonstrated in half or more of the animals. The activity was measured after 0.5 and 4.0 h of dose administration of test compounds. The sign — (mdash) represents an absence of activity at maximum dose administered (300 mg/kg).
[b] Mice were employed in this biological evaluation.
[c] Rats were employed in this biological evaluation.

Table 2
Anticonvulsant evaluation of compounds after oral administration in rats

Compound	Oral administration in rats[a]									
	MES screening					NT screening				
	0.25 h	0.5 h	1 h	2 h	4 h	0.25 h	0.5 h	1 h	2 h	4 h
6	0	0	1	0	1	0	0	0	0	1
11	0	1	0	0	1	0	0	0	1	0
16	0	0	0	1	1	0	0	0	0	1
17	0	0	0	0	1	0	0	0	0	0

[a] The compounds were administered in a dose of 30 mg/kg. The data in the table indicates the number of protected rats out of four.

Table 3
GABA assay for compounds found active in anticonvulsant screening

S. No.	Compound	GABA concentration in μg/100 mg[a]		
		Midbrain	Medulla oblangata	Cerebellum
1	**6**[b]	53.77 ± 2.95	35.48 ± 1.62	28.53 ± 2.66
2	**11**[b]	45.50 ± 3.79	40.42 ± 2.29	27.56 ± 1.69
3	**16**[b]	62.25 ± 2.38	37.74 ± 2.73	31.41 ± 2.15
4	**17**[b]	67.72 ± 1.84	46.62 ± 3.04	29.75 ± 1.63
5	Clobazam[c]	69.51 ± 3.76	47.53 ± 1.96	35.44 ± 2.39
6	Control	35.87 ± 2.65	31.66 ± 4.27	24.68 ± 2.18

[a] Each value represents the mean ± SEM of six rats significantly different from the control at P <0.001.
[b] The compounds were tested at a dose of 100 mg/kg (ip).
[c] The standard drug was tested at dose of 30 mg/kg (ip).

is vital for anticonvulsant activity. These new facts might be expedient in the future research and development of semicarbazones as novel anticonvulsants.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.059.

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15. *Compound* **11**: MP (°C) 235–237; yield 62%; IR (cm^{-1}) (KBr) 3046.2 (aromatic C–H str), 1604.5 and 1502.7 (aromatic C–C str), 1088.3 (C–O of 1,3,4-oxadiazole nucleus), 1670.5 (C=N of 1,3,4-oxadiazole nucleus), 1034.1 (Ar–O–C str), 1671.6 (C=O str of amide), 3435.2 (N–H str of amide), 1626.4 (C=N group), 1522.6 and 1363.8 (N=O str of Ar–NO_2 group); ^{13}C NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 157.7 (C-1′), 118.6 (C-2′), 129.6 (C-3′), 129.2 (C-4′), 127.7 (C-5′), 123.8 (C-6′), 126.6 (C-7′), 126.9 (C-8′), 134.7 (C-9′), 105.9 (C-10′),

72.5 (OCH_2), 172.5 (C-2), 169.7 (C-5), 157.3 (NHCONHNC), 154.8 (NHCONHNC), 17.6 ($NHCONHNCCH_3$), 137.5 (C-1″), 129.9 (C-2″ and C-6″), 123.9 (C-3″ and C-5″), 150.8 (C-4″); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.9–8.2 (m, 11H, ArH), 5.3 (s, 2H, OCH_2), 6.3 (s, 1H, NHCONH), 9.4 (s, 1H, NHCONH), 1.1 (s, 3H, Carbimino CH_3); ESI-MS (methanol) *m/z* 447.5 ($[M+H]^+$).

Compound **16**: Mp (°C) 262–263; yield 64%; IR (cm^{-1}) (KBr) 3041.5 (aromatic C–H str), 1601.8 and 1501.6 (aromatic C–C str), 1093.0 (C–O of 1,3,4-oxadiazole nucleus), 1667.1 (C=N of 1,3,4-oxadiazole nucleus), 1024.8 (Ar–O–C str), 1695.2 (C=O str of amide), 3439.4 (N–H str of amide), 1615.5 (C=N group), 3456.5 (O–H str of alcoholic group), 1174.7 (C–O str of alcoholic group); ^{13}C NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 157.7 (C-1′), 118.7 (C-2′), 129.8 (C-3′), 129.3 (C-4′), 127.8 (C-5′), 123.5 (C-6′), 126.4 (C-7′), 126.9 (C-8′), 134.7 (C-9′), 105.8 (C-10′), 72.3 (OCH_2), 172.7 (C-2), 169.3 (C-5), 157.4 (NHCONHNC), 155.8 (NHCONHNC), 123.8 (C-1″), 130.5 (C-2″ and C-6″), 115.7 (C-3″ and C-5″), 159.8 (C-4″), 131.4 (C-1‴), 129.2 (C-2‴ and C-6‴), 128.7 (C-3‴ and C-5‴), 130.9 (C-4‴); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 6.8–7.7 (m, 16H, ArH), 5.2 (s, 2H, OCH_2), 6.4 (s, 1H, NHCONH), 9.6 (s, 1H, NHCONH), 5.3 (s, 1H, ArOH); ESI-MS (methanol) *m/z* 480.6 ($[M+H]^+$).

Compound **17**: MP (°C) 275–276; yield 61%; IR (cm^{-1}) (KBr) 3040.2 (aromatic C–H str), 1603.5 and 1501.6 (aromatic C–C str), 1086.2 (C–O of 1,3,4-oxadiazole nucleus), 1665.8 (C=N of 1,3,4-oxadiazole nucleus), 1026.8 (Ar–O–C str), 1693.6 (C=O str of amide), 3439.4 (N–H str of amide), 1619.8 (C=N group), 1532.5 and 1359.8 (N=O str of Ar–NO_2 group); ^{13}C NMR (75 MHz, DMSO-d_6, TMS, δ ppm): 157.9 (C-1′), 118.7 (C-2′), 129.6 (C-3′), 129.2 (C-4′), 127.8 (C-5′), 123.6 (C-6′), 126.5 (C-7′), 126.9 (C-8′), 134.8 (C-9′), 105.9 (C-10′), 72.2 (OCH_2), 172.6 (C-2), 169.4 (C-5), 157.3 (NHCONHNC), 155.7 (NHCONHNC), 137.4 (C-1″), 131.1 (C-2″ and C-6″), 123.8 (C-3″ and C-5″), 150.9 (C-4″), 131.3 (C-1‴), 128.8 (C-2‴ and C-6‴), 128.5 (C-3‴ and C-5‴), 130.7 (C-4‴); ^{1}H NMR (300 MHz, DMSO-d_6, TMS, δ ppm): 7.0–8.2 (m, 16H, ArH), 5.3 (s, 2H, OCH_2), 6.3 (s, 1H, NHCONH), 9.6 (s, 1H, NHCONH); ESI-MS (methanol) *m/z* 509.5 ($[M+H]^+$).

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