Synthesis and Biological Screening of 2-Substituted 5,6-Dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles and of Their Intermediates

by **Khalid Mohammed Khan***, **Shagufta Rahat**, **Muhammad Iqbal Choudhary**, **Atta-ur-Rahman**, **Usman Ghani**, **Shahnaz Perveen**, **Shagufta Khatoon**, **Ahsana Dar**, and **Abdul Malik**

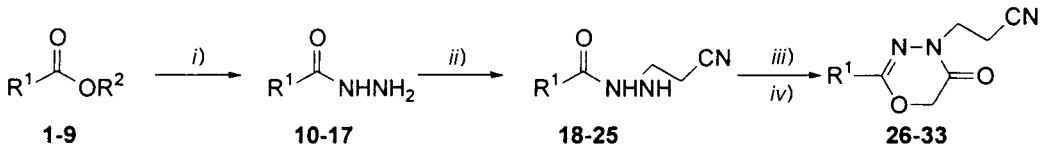
H.E.J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi-75270, Pakistan
(Phone: (92-21)-9243224, 9243211; fax: (92-21)-9243190-91; e-mail:hassaan2@super.net.pk)

To evaluate the effect of substituents on biological activities of electron-rich N-containing heterocycles, the variably 2-substituted 5,6-dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles **26**–**33** were synthesized and evaluated for antibacterial, antifungal, and enzyme-inhibition activities. The target compounds were obtained from alkyl 4- or 3-hydroxy benzoates **1** and **2**, respectively, and from methyl indoleacetate **3**. The phenolic OH group of benzoates **1** and **2** were substituted with *p*-toluenesulfonyl (→**4** and **5**), benzoyl (→**6** and **7**), and benzyl groups (→**8** and **9**) and then converted to 5,6-dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles. To establish structure-activity relationships (SAR), a pharmacological screening of the intervening intermediates was also conducted, which revealed that the intermediate hydrazide **11** possesses significant antimicrobial and MAO-A inhibiting properties and intermediates **12**, **24**, **28**, and **29** appreciable antifungal activities. Compound **7** inhibits α-chymotrypsin.

Introduction. – A diversity of biological effects is associated with 1,3,4-oxadiazine and its derivatives, they exhibit cardiovascular [1–3], antibacterial [4], plant-growth regulating [5], miticidal and nematocidal [6], acricidal [7], and insecticidal activities [8] and monoamine-oxidase (MAO) inhibition [9]. The promising therapeutic potential of this class of compounds prompted us to synthesize and biologically screen a series of structural variants of 4*H*-1,3,4-oxidiazine-5(6*H*)-ones. To establish structure activity relationships (SAR), the activities of the intervening intermediates were also determined. Thus, the readily available alkyl benzoates **1**, **2**, and **4**–**9** and the indoleacetate **3** were transformed to the target 5,6-dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles **26**–**33** in excellent yields. The structures of **26**–**33** were established by spectroscopic techniques, and their biological activities were determined.

Results and Discussion. – The synthesis of the 5,6-dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles **26**–**33** is outlined in the *Scheme*. The phenolic OH group of the benzoates **1** and **2** was substituted with *p*-toluenesulfonyl (→**4** and **5**), benzoyl (→**6** and **7**) and benzyl groups (→**8** and **9**). The benzoates **1**, **2**, and **4**–**9**, and the indoleacetate **3** were then treated with hydrazine hydrate in EtOH to afford the hydrazides **10**–**17**. Alkylation of a hydrazide occurs at the N(1) or N(2) atom depending upon the conditions; thus, in neutral medium, the terminal N(2) atom [10] is alkylated, whereas, in the presence of a strong base like Na or NaOMe, the position of alkylation is strongly dependent on the nature of the solvent [11]: aprotic solvents like

Scheme



	R^1	R^2
1	HO–	CH_3
2	HO–	C_2H_5
3	CH_2– (N–H)	CH_3
4	TsO–	CH_3
5	TsO–	C_2H_5
6	BzO–	CH_3
7	BzO–	C_2H_5
8	BnO–	CH_3
9	BnO–	C_2H_5

	R^1
10, 18, 26	HO–
11, 19, 27	TsO–
12, 20, 28	BzO–
13, 21, 29	BnO–
14, 22, 30	TsO–
15, 23, 31	BzO–
16, 24, 32	BnO–
17, 25, 33	CH_2– (N–H)

i) N_2H_4, H_2O, EtOH, reflux, 48 h. *ii*) $H_2C{=}CHCN$, EtOH, reflux, 48 h. *iii*) $ClCH_2COCl$, $CHCl_3$, reflux, 1 h. *iv*) K_2CO_3, EtOH, reflux, 18–24 h.

Et_2O and benzene favor N(1) substitution, while, in protic solvents like EtOH, N(2) substitution predominates. *Michael* reaction between hydrazides **10**–**17** and acrylonitrile led exclusively to N(2)-substituted derivates **18**–**25**. Under no circumstances was the formation of double *Michael* products detectable. The cyclization of the 2-(2-cyanoethyl)hydrazides **18**–**25** was brought about with chloroaectyl chloride in the presence of K_2CO_3 to furnish the target 5,6-dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles **26**–**33** in excellent yields.

All compounds synthesized were assessed for enzyme-inhibitory activity against monoamine oxidase (MAO), serine proteases (α-chymotrypsin, trypsin, and elastase), cysteine protease papain, and tyrosinase enzymes. Significant inhibition was observed for monoamine oxidase and α-chymotrypsin; the other enzymes were found to be either resistant or only partially sensitive to the compounds tested.

MAO Activities in the absence of test compounds (control enzyme) were found to be 18 ± 1.7 nmol of H_2O_2 h^{-1} mg^{-1} of protein. This activity was inhibitied by hydrazide **11** in a concentration-dependent manner and similarly to the monoamine oxidase A (MAO-A) inhibitors clorgyline and moclobemide [12]; clorgyline, moclobemide, and **11** caused complete enzyme inhibition at 0.1, 10000, and 100 μM, respectively (*Fig. 1*). The inhibitor concentrations required for 50% inhibition of enzyme (IC_{50}) decreased in the order moclobemide > hydrazide **11** > clorgyline (*Table 1*). Although **11** was *ca.* 60 times less potent than clorgyline (a nonselective inhibitor of MAO-A); it was *ca.* 80 times more potent than moclobemide, a clinically effective, selective inhibitor of MAO-A. This suggests that hydrazide **11** may have potential for future research aimed at the synthesis of a better antidepressant agent. On the other hand, compound **33** failed to inhibit enzyme activity up to the highest tested concentration (100 μM). Since hydrazide **10** containing a *p*-OH group and hydrazide **14** bearing a *m*-TsO group failed to inhibit MAO, these groups do probably not interfere with the enzyme. The dramatic inhibition of MAO by hydrazide **11** containing a *p*-TsO group suggests that this group is likely to be responsible for the observed interaction with the enzyme. However, the conversion of the hydrazide to the oxadiazine ring (**11** → **27**) resulted in complete loss of activity, implying that the N(2) atom of the hydrazide plays a crucial role. Thus, simultaneous occurrence of a hydrazide moiety and a *p*-TsO group at a phenyl ring are prerequisites for MAO inhibition.

The most potent among the inhibitors of α-chymotrypsin was benzoate **7** containing a *m*-BzO group ($IC_{50} = 29.34 \pm 1.15$ μM), while other compounds of the series showed moderate to weak activity (*Table 2*, *Fig. 2*). The active site of α-chymotrypsin contains a primary specificity pocket that interacts with the bulky side chains of the amino acids tyrosine, phenylalanine, and tryptophan, suggesting that the inhibitory activity of **7** might be due to its aryl rings. To determine which aryl ring of **7** is critical for inhibition, its inhibitory activity was compared with that of benzoate **5** containing a *m*-TsO group. A significant decrease in the activity was observed in the latter case (see *Table 2* and *Fig. 2*).

A further decrease of α-chymotrypsin inhibition was observed when the hydrazide moiety was converted to a 2-(2-cyanoethyl)hydrazide function. Moreover, none of the target oxadiazine derivatives were significantly active, probably due to affinity or steric factors. Thus, the presence of a *m*-BzO group at the benzoate plays an important role in the inhibition of α-chymotrypsin.

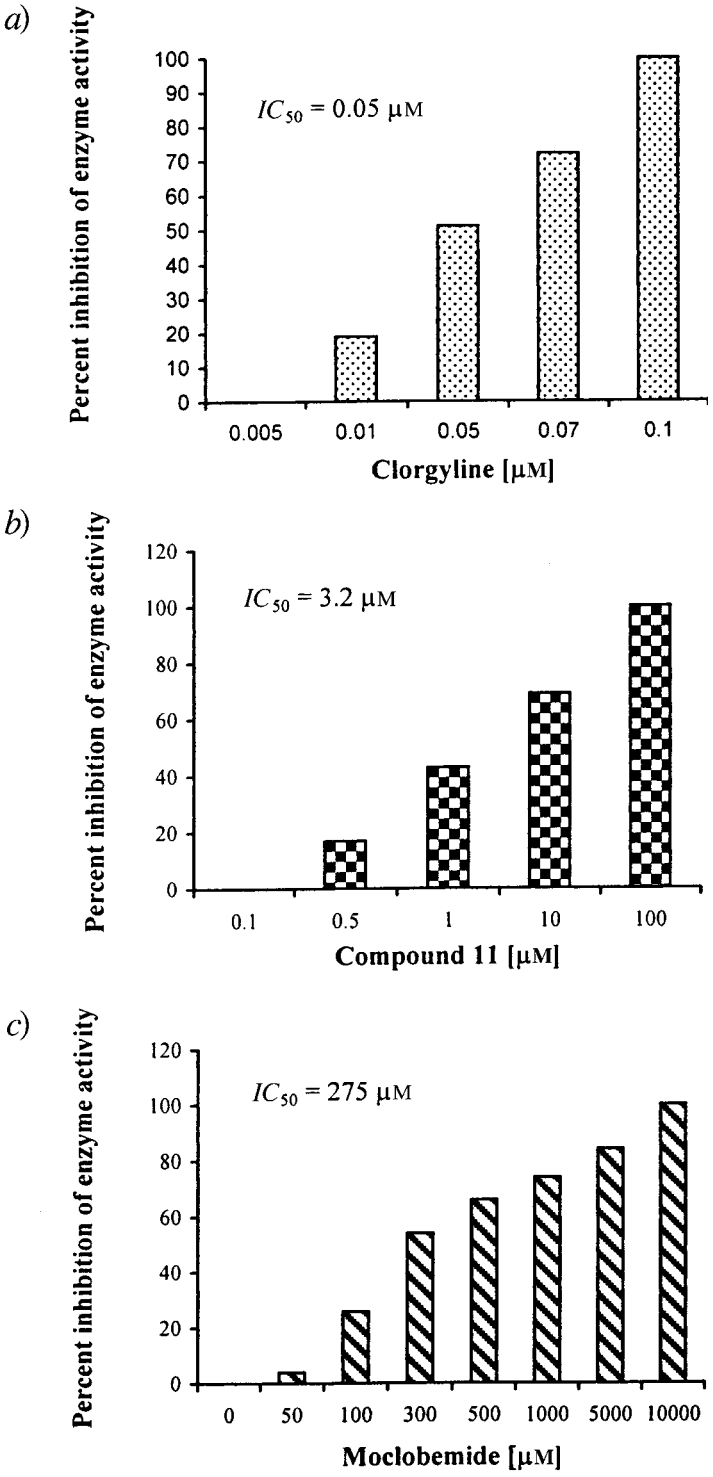


Fig. 1. *Effect of various concentrations of the inhibitors* a) *clorgyline*, b) *hydrazide* **11**, *and* c) *moclobemide on MAO-A activity.* A sample of synaptosomes (28 mg/ml protein) was pre-incubated with the inhibitor for 20 min at 37° before the addition of 5-hydroxytryptamine (500 μM). Inhibition of enzyme activity is expressed as % of remaining activity of the control sample pre-incubated without the inhibitor. Each bar is the mean of 2–6 determinations, each in duplicate or triplicate.

Table 1. IC_{50} *Values of Monoamine-Oxidase Inhibitors* (clorgyline and moclobemide) *and of Compound* **11**

	Clorgyline	Moclobemide	**11**
IC_{50} [μM]	0.05	275	3.2

Table 2. *Inhibition of α-Chymotrypsin by 2-Substituted 5,6-Dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles and of Their Intermediates*

	IC_{50} [μM]	Percentage inhibition at 1 μM	Percentage inhibition at 100 μM
7	29.34 ± 1.15	–	–
22	1160 ± 37.12	–	–
21	–	77.11	–
25	–	45.92	–
33	–	48.68	–
4	–	–	35.16
5	-	–	64.40
6	–	–	58.47
8	–	–	27.63
27	–	–	28.81
32	–	–	26.31

Antibacterial activity of various synthesized compounds was tested against six *Gram*-positive and four *Gram*-negative bacterial strains (*Table 3*). The results were compared with the standard drugs amoxillin and ampicillin. Hydrazide **11** having a *p*-TsO substituent at the phenyl ring was found to be the most active against a broad range of both *Gram*-positive and *Gram*-negative bacteria. Interestingly, hydrazide **10** having a *p*-OH group showed significant activity against *Pseudomonas aeroginosa* and *Salmonella typhi* and a modest activity against *Shigella boydii* and *Streptococcus pyogenes.* The corresponding 2-(2-cyanoethyl)hydrazide **18** showed varying activities against *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeroginosa*, *S. typhi*, *S. aureus*, and *S. pyogenes*, and the corresponding 4*H*-1,3,4-oxadiazine derivative **26** displayed weak activity against *P. mirabilis*, *S. typhi*, *S. boydii*, *S. aureus*, and *S. pyogenes* but was significantly active against *K. pneumoniae*, *P. aeroginosa*, and *B. cerous.* Compounds **12**, **20**, and **28** all having a *p*-BzO group, predominantly exhibited weak to moderate activity against some of the bacterial strains. Hydrazide **13** demonstrated moderate activity against *K. pneumoniae*, *P. mirabilis*, *P. aeroginosa*, *S. typhi*, *S. boydii*, *S. aureus*, and *S. pyogenes*, while its cyanoethyl derivative **21** displayed the same degree of activity against *E. coli*, K. *pneumoniae*, *P. aeroginosa*, *S. typhi*, *S. boydii*, and *S. aureus*, and no activity against *P. mirabilis*, *P. cerous*, *C. diptheriae*, and *S. pyogenes* as compared to its precursor. The corresponding 4*H*-oxadiazine derivative **29** failed to show activity against *E. coli*, *P. mirabilis*, *S. boydii*, *B. cerous*, *S. aureus*, and *S. pyogenes*, moderately active against *K. pneumoniae*, P. *aeroginosa*, and *C. diptheriae*, and significant activity against *S. typhi*. Compounds **19** and **27**, the 2-(2-cyanoethyl)hydrazide and oxadiazine derivatives of hydrazide **11**, respectively, were less active compared with **11**. The important bactericidal activity of **11** could be related to the strong electron-withdrawing nature of the *p*-TsO substituent at the phenyl ring. In general, poor activity of the oxadiazine derivatives is possibly due to the steric factors being responsible for retarding passive diffusion of the molecule into the bacterial cell. It has been suggested

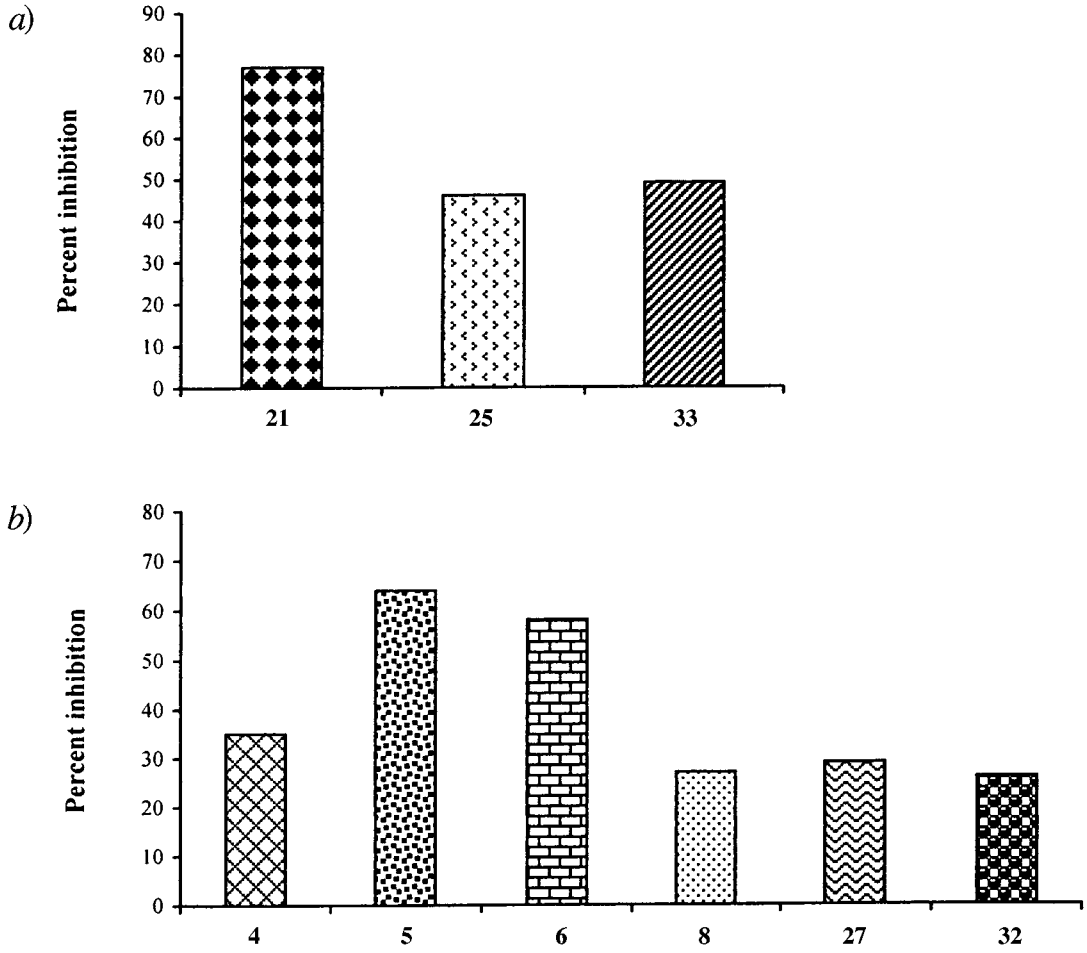


Fig. 2. *Effect of various compounds as inhibitors of α-chymotrypsin:* a) *1* μM *of* **21**, **25**, *and* **33** *and* b) *100* μM *of* **4**, **5**, **6**, **8**, **27**, *and* **32**

that less substitution at an aromatic ring promotes passive diffusion. The electronic nature of the substituent at the *p*-position and steric factors promotes passive diffusion. The electronic nature of the substituent at the *p*-position and steric factors associated with the six-membered oxadiazine ring thereby may be responsible for the poor bactericidal activity.

Hydrazide **11** containing a *p*-TsO group was the most potent bactericidal agent as compared to its parent Me ester **4** and all the tested compounds. However, alkylation at the N(2)-atom of the hydrazide moiety attenuated the antibacterial activity, revealing that the N(2)-atom of the hydrazide moiety is indispensable for the bacterial inhibition. Cyclization caused a further decline in the antibacterial activity, indicating that the H-atom at N(2) in open-chain compounds is probably responsible for it.

Table 3. *Antibacterial Activity of 2-Aryl-Substituted 5,6-Dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles and of Their Intermediates*[a])

	4	**6**	**10**	**11**	**12**	**13**	**18**	**19**	**20**	**21**	**26**	**27**	**28**	**29**
Gram-negative:														
Escherichia coli	7.0	7.0	0.5	11	000.5	9.0	00	6.0	7.0	5.0	0.5	7.0	0.5	
Klebsiella pneumoniae	00	00	0.5	12	7.0	6.0	8.0	6.0	00	7.0	8.0	6	6.0	6.0
Proteus mirabilis	00	00	00	10	00	6.0	00	8.0	00	00	0.5	00	0.5	00
Pseudomonas aeroginosa	00	6.0	9.0	12	00	6.0	7.0	6.0	7.0	7.0	8.0	0.5	7.0	7.0
Salmonella typhi	8.0	00	8.0	11	00	7.0	6.0	00	00	6.0	0.5	6.0	7.0	8.0
Shigella boydii	00	00	6.0	10	00	6.0	0.5	6.0	00	7.0	0.5	7.0	7.0	0.5
Gram-positive:														
Bacillus cerous	6.0	00	00	10	00	0.5	0.5	00	00	00	8.0	0.5	00	0.5
Corynebacterium diptheriae	6.0	8.0	0.5	11	00	0.7	00	00	8.0	00	6.0	00	00	6.0
Staphylococcus aureus	7.0	7.0	00	9.0	7.0	6.0	6.0	6.0	6.0	6.0	00	6.0	00	00
Streptococus pyogenes	00	5.5	7.0	11	7.0	7.0	6.0	6.0	00	00	00	8.0	0.5	00

[a]) <5: weak; 5–7: moderate; >7: significant.

The 2-aryl-substituted 5,6-dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles and their synthetic intermediates were also tested for their *in vitro* antifungal activity against the eleven fungal strains *Aspergillus flavus*, *Trichophyton schoenleinii*, *Trichophyton rubrum*, *Pseudallescheria boydii*, *Candida albicans*, *Aspergillus niger*, *Microsporum canis*, *Tricophyton simii*, *Trichophyton mentagrophytes*, *Fusarium oxysporum*, and *Fusarium solani* (see *Table 4*). The results were compared with the standard drugs micoanazole, ketocanozole, benlate, and nabam. Hydrazide **11** having a *p*-TsO substituent at the phenyl ring showed strong growth-inhibitory activity against *C. albicans* and *P. boydii* in an agar-dilution assay, indicating that it can be considered a lead for design of drug against candidiasis. Hydrazide **12** containing a *p*-BzO group showed significant activity against *P. boydii* and *F. oxysporum*, whereas **24** containing a *m*-BnO group and a side chain at the N(2) atom was active against *P. boydii*. The *p*-BzO-substituted 4*H*-1,3,4-oxadiazine derivative **28** appeared to be active against *T. simii*, whereas the *p*-BnO-substituted 4*H*-1,3,4-oxadiazine derivative **29**, showed activity against *M. canis*. In general, the oxadiazine derivatives were very weakly active.

Table 4. *Antifungal Activity of 2-Aryl-Substituted 5,6-Dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles and of Their Intermediates*

	4	**6**	**8**	**10**	**11**	**12**	**13**	**18**	**19**	**20**	**21**	**24**	**26**	**27**	**28**	**29**
Aspergillus flavus	00	00	00	00	00	57	00	00	00	00	00	58	00	00	00	00
Trichophton schoenleinii	16	00	33	50	00	00	3.0	42	00	00	61	00	50	50	00	34
Trichophyton rubrum	00	00	00	56	00	00	00	00	7.0	00	00	00	67	33	00	00
Pseudallescheria boydii	00	00	00	33	82	95	00	00	00	00	00	100	00	45	00	00
Candida albicans	00	00	00	7.0	100	7.0	00	00	00	00	00	15	00	00	00	00
Aspergillus niger	12	00	36	00	3.0	9.0	00	14	00	00	7.0	6.0	50	00	00	15
Microsporum canis	00	00	50	00	6.0	52	00	33	00	00	54	00	71	14	00	88
Trichopyton simii	25	00	5.0	50	00	00	00	15	00	00	38	00	30	60	100	30
Trichophytonmentagrophytes	00	00	00	00	00	29	00	00	00	00	00	00	00	00	00	00
Fusarium oxysporum	50	00	50	00	29	83	50	50	00	00	50	19	25	14	7	30
Fusarium solani	30	00	48	00	00	31	60	56	00	00	54	58	00	25	12	30

Conclusions. – The structure-activity relationship (SAR) study conducted with 2-aryl-substituted 5,6-dihydro-5-oxo-4*H*-1,3,4-oxadiazine-4-propanenitriles for selected biological activities indicated that these compounds were slightly active, in contrast to our hypothesis. Interestingly, some intermediates of their syntheses exhibited biological activities. Particularly, hydrazide **11** bearing a *p*-TsO group demonstrated significant monoamine-oxidase inhibition and bactericidal properties, suggesting that the electron-withdrawing nature of *p*-TsO probably enhances the interaction with the active sites of the enzyme and its entry into the bacterial cell. Compounds **11**, **12**, **24**, **28**, and **29** demonstrated excellent antifungal activities against a few fungal strains. The *m*-BzO-substituted benzoate strongly inhibited α-chymotrypsin, the *m*-BzO group in this type of ester probably playing an important role in the inhibition process. Possibilities also exist for further exploring the structure-activity relationships of these compounds.

Experimental Part

General. Anh. $CHCl_3$ and EtOH were prepared by standard methods. All other solvents and reagents were reagent grade and used directly without purification, except for chloroacetyl chloride and benzoyl chloride, which were distilled before use. M.p.: open capillaries, *Büchi-535* apparatus; uncorrected. Column chromatography: silica gel (70–230 mesh, *E. Merck*). IR: *Shimadzu IR-460* for KBr pellets and *Jasco A-302* spectrophotometer for $CHCl_3$ solns.; in cm^{-1}. ^{1}H-NMR: *Bruker* apparatus; at 300 and 400 MHz; δ in ppm rel. to $SiMe_4$ (=0 ppm) as internal standard. EI-MS: *Finnigan-MAT 311-A* apparatus; *m*/*z* (rel. %).

Preparation of Synaptosomes. Adult *Wistar* rats (both sexes, locally bred) were sacrificed by rapid decapitation, followed by quick removal of brains. Homogenate was prepared in 20 volumes of 0.32M ice-cold sucrose by means of polytron (8000 $min^{-1} \times 8$ for 3 s). The homogenate was centrifuged at 1000 *g* for 10 min at 4°, and the supernatant was further centrifuged at 17000 *g* for 30 min. The pellet obtained was resuspended in 10 volumes of sucrose and homogenized by means of polytron as described above. Synaptosomes were used either for the determination of MAO-A activity immediately or aliquoted and stored at −50° till further use [13].

Monoamine Oxidase Assay: MAO-A Activity was determined by the fluorometric method [14][15] with 5-hydroxytryptamine (500 μM) as a substrate. Synaptosomes (20 μl) were pre-incubated either with buffer or test compound (**11** or **33** in 10% DMSO in *d*/*w*) for 30 min at 37°, followed by the addition of substrate (50 μl). Assay tubes were incubated for 20 min with a final reaction volume of 200 μl. An adrenaline/peroxidase system was used to form fluorescent adrenolutine; the intensity of the fluorescence was determined at an emission wavelength of 550 nm with an excitation wavelength of 405 nm. In all of the experiments, H_2O_2 (2 nmol) was used as a standard that gave a fluorescence of 251 ± 3.5. The control enzyme activity was expressed in nmol of H_2O_2 formed h^{-1} mg^{-1} protein. The % inhibition of enzyme activity in the presence of test compound was determined by comparing it with the control value. Clorgyline (an irreversible MAO-A inhibitor) at 0.1 μM caused complete enzyme inhibition, confirming the presence of MAO-A in the reaction mixture. Protein concentrations were determined by the method of *Lowry et al*. [16] with bovine serum albumin as a standard.

Chymotrypsin-Inhibition Assay. Protease inhibitory activity of selected synthesized compounds was determined according to [17]. Increasing concentrations of compounds were incubated with α-chymotrypsin (9 U/ml) in 50 mM *Tris*·HCl buffer (pH 7.6) at 25° for 30 min. Release of 4-nitroaniline was continuously monitored at 405 nm in a *SprctraMax-340* microplate reader (*Molecular Devices*, USA) immediately after the addition of 50 μl of substrate *N*-succinyl-phenylalanine-4-nitroanilide (2 mg/ml). Results were compared with chymostatin, a standard inhibitor of chymotrypsin [18]. The % inhibition was calculated as the difference of absorbance values with and without compounds (see *Table 2*).

Antibacterial Activity. The synthesized compounds were tested against six *Gram*-negative (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeroginosa*, *S. typhi*, and *S. boydii*) and four *Gram*-positive (*B. cerous*, *C. diptheriae*, *S. aureus*, and *S. pyogenes*) bacterial strains by the agar-well-diffusion method [19]. The wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm apart. Two- to eight-hours-old bacterial inocula containing approximately 10^4–10^6 colony-forming units (CFU)/ml were spread on the surface of nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (2 mg/ml in DMSO) was introduced in respective wells. Other wells supplemented with DMSO and reference antibacterial drugs served as negative and positive controls, respectively. The plates were incubated immediately

at 37° for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control.

Antifungal Activity. The synthesized compounds were tested for antifungal activity against six human pathogens (*A. flavus*, *T. schoenleinii*, *T. rubrum*, *P. boydii*, *C. albicans*, and *A. niger*), three animal pathogens (*M. canis*, *T. simii*, and *T. mentagrophytes*), and two plant pathogens (*F. oxysporum* and *F. solani*) by means of the tube diffusion test [20]. Micoanazole (75 μg/ml), ketocanozole (75 μg/ml), benlate (100 μg/ml), and nabam (50 μg/ml) were used as standard drugs. Stock solns. of pure compounds (12 μg/ml) were prepared in sterile DMSO. Sabouraud dextrose agar was prepared by mixing sabouraud (32.5 g), glucose agar (4%) and agar-agar (4 g) in 500 ml of distilled H_2O followed by steam dissolution; the media (4 ml) was dispensed into screw-caped tubes and autoclaved at 121° for 15 min. Test compound (66.6 μl) was added from the stock soln. to non-solidified sabouraud agar media (50°). Tubes were allowed to solidify at r.t. and inoculated with 4-mm diameter of inocula derived from seven-days-old respective fungal culture. For nonmycelial growth, an agar surface streak was employed. The tubes incubated at 27–29° for 7–10 days and the growth in the compound-containing media was determined by measuring the linear growth (mm) and growth inhibition with reference to the respective control.

Benzoates **4** *and* **5**: *General Procedure.* TsCl (0.04 mol) was added in small portions to a cold soln. of **1** or **2** (0.03 mol) in pyridine (0.06 mol) at $<10°$. After stirring for 3 h at $<10°$, the mixture was kept overnight at 5°, neutralized with 2M HCl, and filtered. The solid was washed with H_2O and recrystallized from MeOH: **4** or **5**, resp.

Methyl 4-{[(4-Methylphenyl)sulfonyl]oxy}benzoate (**4**): Yield 89%. M.p. 85°. IR (KBr): 3000, 1725, 1440. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.42 (*s*, 3 H); 3.82 (*s*, 3 H); 7.12–7.95 (*m*, 8 arom. H). Anal. calc. for $C_{15}H_{14}O_5S$ (306.24): C 58.83, H 4.61; found: C 58.73, H 4.59.

Ethyl 3-{[(4-Methylphenyl)sulfonyl]oxy}benzoate (**5**): Yield 79%. M.p. 37°. IR (KBr): 3000, 1725, 1375. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.34 (*t*, 3 H); 2.43 (*s*, 3 H); 4.32 (*q*, 2 H); 7.32–7.93 (*m*, 8 arom. H). Anal. calc. for $C_{16}H_{16}O_5S$ (320.27): C 60.00, H 5.03, found: C 59.91, H 5.00.

Benzoates **6** *and* **7**: *General Procedure.* To a mixture of **1** or **2** (0.03 mol) in pyridine (0.06 mol), benzoyl chloride (0.05 mol) was added dropwise and the mixture stirred overnight. The mixture was then poured in crushed ice and acidified with dil. HCl soln. The resulting crystalline solid was filtered, washed with MeOH (20 ml) and H_2O (20 ml), and air-dried: **6** or **7**, resp.

Methyl 4-(Benzoyloxy)benzoate (**6**): Yield 73%. M.p. 126°. IR (KBr): 3000, 1725, 1680. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.81 (*s*, 3 H); 7.41–8.13 (*m*, 9 arom. H). Anal. calc. for $C_{15}H_{12}O_4$ (256.25): C 70.30, H 4.72; found: C 70.24, H 4.68.

Ethyl 3-(Benzoyloxy)benzoate (**7**): Yield 80%. M.p. 52°. IR (KBr): 3000, 1720, 1680. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.33 (*t*, 3 H), 4.35 (*q*, 2 H); 7.61–8.02 (*m*, 9 arom. H). Anal. calc. for $C_{16}H_{14}O_4$ (270.30): C 71.10, H 5.22; found: C 71.08, H 5.18.

Benzoates **8** *and* **9**. *General Procedure.* To a mixture of **1** or **2** (0.02 mol) and K_2CO_3 (0.04 mol) in acetone (30 ml), benzyl bromide (0.02 mol) was added dropwise at r.t. The mixture was refluxed at 60° for 3 h and then filtered. The filtrate was evaporated and the residue crystallized from hot hexane: **8** or **9**, resp.

Methyl 4-(Benzyloxy)benzoate (**8**): Yield 62%. M.p. 94°. IR (KBr): 3300, 1725, 1600. ^{1}H-NMR (300 MHz, (D_6)DMSO): 3.75 (*s*, 3 H); 5.14 (*s*, 2 H); 7.12–7.91 (*m*, 9 arom. H). Anal. calc. for $C_{15}H_{14}O_3$ (242.27): C 74.37, H 5.82; found: C 74.32, H 5.79.

Ethyl 3-(Benzyloxy)benzoate (**9**): Yield 82%. M.p. 69°. IR (KBr): 3300, 1725, 1600. ^{1}H-NMR (300 MHz, (D_6)DMSO): 1.32 (*t*, 3 H); 3.82 (*s*, 3 H); 5.25 (*s*, 2 H); 4.32 (*q*, 2 H); 7.21–7.65 (*m*, 9 arom. H). Anal. calc. for $C_{16}H_{16}O_3$ (256.30): C 74.98, H 5.82; found: C 74.93, H 5.75.

Hydrazides **10**–**17**: *General Procedure.* To 99% hydrazine hydrate (0.01 mol) in EtOH (3.7 ml), **1** or one of **3**–**9** (0.06 mol) was added in small portions and then heated under reflux for 28 h. After evaporation, the oily residue crystallized overnight in the cold.

4-Hydroxybenzoic Acid Hydrazide (**10**): Yield 80%. M.p. 268°. IR (KBr): 3350, 3000, 1620. ^{1}H-NMR (300 MHz, (D_6)DMSO): 6.75–7.62 (*dd*, 4 H); 9.52 (*s*, 1 H). Anal. calc. for $C_7H_8N_2O_2$ (152.15): C 55.26, H 5.3, N 18.41; found: C 55.22, H 5.32, N 18.37.

4-{[(4-Methylphenyl)sulfonyl]oxy}benzoic Acid Hydrazide (**11**): Yield 63%. M.p. 105°. IR (KBr): 3350, 1690, 1640, 1380. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.38 (*s*, 3 H); 6.75–7.90 (*m*, 8 arom. H); 9.49 (*s*, 1 H). Anal. calc. for $C_{14}H_{14}N_2O_4S$ (306.24): C 54.90, H 4.60, N 9.14; found: C 54.87, H 4.51, N 9.11.

4-(Benzoyloxy)benzoic Acid Hydrazide (**12**): Yield 69%. M.p. 255°. IR (KBr): 3350, 1700, 1690, 1640. ^{1}H-NMR (300 MHz, (D_6)DMSO): 6.80–7.68 (*m*, 9 arom. H); 9.46 (*s*, 1 H). Anal. calc. for $C_{14}H_{12}N_2O_3$ (256.26): C 65.61, H 4.72, N 10.93; found: C 65.60, H 4.68, N 10.90.

4-(Benzyloxy)benzooic Acid Hydrazide (**13**): Yield 73%. M.p. 140°. IR (KBr): 3290, 1680, 1600. ^{1}H-NMR (300 MHz, (D_6)DMSO): 5.15 (*s*, 2 H); 7.03–7.85 (*m*, 9 arom. H); 9.38 (*s*, 1 H). Anal. calc. for $C_{14}H_{14}N_2O_2$ (242.27): C 69.40, H 5.82, N 11.56; found: C 69.37, H 5.84, N 11.47.

3-{[(4-Methylphenyl)sulfonyl]oxy}benzoic Acid Hydrazide (**14**): Yield 72%. M.p. 155°. IR (KBr): 3300, 1660, 1610, 1375. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.42 (*s*, 3 H); 7.16–7.90 (*m*, 8 arom. H); 9.91 (*s*, 1 H). Anal. calc. for $C_{14}H_{14}N_2O_4S$ (306.24): C 54.90, H 4.60, N 9.14; found: C 54.87, H 4.52, N 9.17.

3-(Benzoyloxy)benzoic Acid Hydrazide (**15**): Yield 60%. M.p. 154°. IR (KBr): 3300, 1710, 1680, 1620. ^{1}H-NMR (300 MHz, (D_6)DMSO): 7.62–8.15 (*m*, 9 arom. H); 9.65 (*s*, 1 H). Anal. calc. for $C_{14}H_{12}N_2O_3$ (256.26): C 65.62, H 4.72, N 10.93; found: C 65.63, H 4.67, N 10.90.

3-(Benzyloxy)benzoic Acid Hydrazide (**16**): Yield 85%. M.p. 127°. IR (KBr): 3300, 1640, 1610. ^{1}H-NMR (300 MHz, (D_6)DMSO): 5.21 (*s*, 2 H); 7.25–7.63 (*m*, 9 arom. H); 9.82 (*s*, 1 H). Anal. calc. for $C_{14}H_{14}N_2O_2$ (242.27): C 69.40, H 5.82, N 11.56; found: C 69.29, H 5.86, N 11.49.

1H-Indole-3-acetic Acid Hydrazide (**17**): Yield 68%. M.p. 137°. IR (KBr): 3300, 1640, 1620. ^{1}H-NMR (300 MHz, (D_6)DMSO): 4.27 (*s*, 2 H); 6.90–7.58 (*m*, 5 arom. H); 9.81 (*s*, 1 H). Anal. calc. for $C_{10}H_{11}N_3O$ (189.21): C 63.48, H 5.86, N 22.21; found: C 63.41, H 5.80, N 22.17.

2-(2-Cyanoethyl)hydrazides **18**–**25**: *General Procedure*. To one of the hydrazides **10**–**17** (0.5 mol) in EtOH (172 ml), acrylonitrile (1.5 mol) was added and the mixture heated under reflux for 48 h. After evaporation, the residue was chromatographed (silica gel, hexane/AcOEt 7:3): crystalline **18**–**25**.

4-Hydroxybenzoic Acid 2-(2-Cyanoethyl)hydrazide (**18**): Yield 75%. M.p. 135°. IR (KBr): 3500, 3350, 2350, 1660, 1610. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.62 (*t*, 2 H); 3.05 (*t*, 2 H); 6.90–7.74 (*dd*, 4 H); 10.0 (*s*, 1 H). Anal. calc. for $C_{10}H_{11}N_3O_2$ (205.21): C 58.53, H 5.40, N 20.48; found: C 58.46, H 5.28, N 20.56.

4-{[(4-Methylphenyl)sulfonyl]oxy}benzoic Acid 2-(2-Cyanoethyl)hydrazide (**19**): Yield 61%. M.p. 103°. IR (KBr): 3300, 2240, 1690, 1620. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.40 (*s*, 3 H); 2.62 (*t*, 2 H); 3.05 (*t*, 2 H); 7.15–7.82 (*m*, 8 arom. H); 10.1 (*d*, 1 H). Anal. calc. for $C_{17}H_{17}N_3O_4S$ (359.31): C 56.83, H 4.77, N 11.70; found: C 56.84, H 4.69, N 11.71.

4-(Benzoyloxy)benzoic Acid 2-(2-Cyanoethyl)hydrazide (**20**): Yield 50%. M.p. 128°. IR (KBr): 3300, 2230, 1725, 1670, 1625. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.63 (*t*, 2 H); 3.05 (*t*, 2 H); 7.15–7.82 (*m*, 9 arom. H); 10.1 (*d*, 1 H). Anal. calc. for $C_{17}H_{15}N_3O_3$ (309.32): C 66.01, H 4.89, N 13.59; found: C 66.03, H 4.81, N 13.61.

4-(Benzyloxy)benzoic Acid 2-(2-Cyanoethyl)hydrazide (**21**): Yield 66%. M.p. 139°. IR (KBr): 3310, 2240, 1680, 1630. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.62 (*t*, 2 H); 3.0 (*t*, 2 H); 5.15 (*s*, 2 H); 7.05–7.85 (*m*, 9 arom. H); 10.01 (*d*, 1 H). Anal. calc. for $C_{17}H_{17}N_3O_2$ (295.34): C 69.14, H 5.80, N 14.23; found: C 69.10, H 5.86, N 14.17.

3-{[(4-Methylphenyl)sulfonyl]oxy}benzoic Acid 2-(2-Cyanoethyl)hydrazide (**22**): Yield 64%. M.p. 39°. IR (KBr): 3300, 2300, 1680, 1600, 1375. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.42 (*s*, 3 H); 2.6 (*t*, 2 H); 3.0 (*t*, 2 H); 7.2–7.91 (*m*, 8 arom. H); 10.1 (*s*, 1 H). Anal. calc. for $C_{17}H_{17}N_3O_4S$ (359.31): C 56.83, H 4.77, N 11.70; found: C 56.80, H 4.81, N 11.67.

3-(Benzoyloxy)benzoic Acid 2-(2-Cyanoethyl)hydrazide (**23**): Yield 55%. M.p. 192°. IR (KBr): 3350, 2230, 1670, 1600. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.62 (*t*, 2 H); 2.91 (*t*, 2 H); 6.90–8.05 (*m*, 9 arom. H); 10.15 (*s*, 1 H). Anal. calc. for $C_{17}H_{15}N_3O_3$ (309.32): C 66.01, H 4.89, N 13.59; found: C 65.96, H 4.87, N 13.58.

3-(Benzyloxy)benzoic Acid 2-(2-Cyanoethyl)hydrazide (**24**): Yield 72%. M.p. 68°. IR (KBr): 3300, 2240, 1680, 1620. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.62 (*t*, 2 H); 3.25 (*t*, 2 H); 5.15 (*s*, 2 H); 7.15–7.55 (*m*, 9 arom. H); 10.1 (*d*, 1 H). Anal. calc. for $C_{17}H_{17}N_3O_2$ (295.34): C 69.14, H 5.80, N 14.23; found: C 69.12, H 5.75, N 14.19.

1H-Indole-3-acetic Acid 2-(2-Cyanoethyl)hydrazide (**25**): Yield 75%. M.p. 123°. IR (KBr): 3300, 2250, 1660, 1620. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.62 (*t*, 2 H); 3.05 (*t*, 2 H); 4.21 (*s*, 2 H); 6.90–7.50 (*d*, 1 H); 10.01 (*s*, 1 H). Anal. calc. for $C_{13}H_{14}N_4O$ (242.28): C 64.45, H 5.82, N 23.13; found: C 64.41, H 5.75, N 23.09.

5,6-Dihydro-5-oxo-4H-1,3,4-oxadiazine-4-propanenitriles **26**–**33**: *General Procedure*. To a soln. of one of the (cyanoethyl)hydrazides **18**–**25** (0.01 mol) in anh. $CHCl_3$ (135 ml), chloroacetyl chloride (0.02 mol) was added dropwise, and the mixture was refluxed for 3 h. After cooling and evaporation, the residue was dissolved in anh. EtOH (120 ml), K_2CO_3 (0.04 mol) added, and the mixture refluxed overnight under stirring. Filtration and evaporation gave, in each case, an oily residue, which was chromatographed (silica gel, hexane/AcOEt 7:3): **26**–**33**.

5,6-Dihydro-2-(4-hydroxyphenyl)-5-oxo-4H-1,3,4-oxadiazine-4-propanenitrile (**26**): Yield 67%. M.p. 209°. IR (KBr): 3500, 2360, 1660. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.90 (*t*, 2 H); 3.91 (*t*, 2 H); 4.80 (*s*, 2 H); 6.82–7.65 (*dd*, 4 arom. H). Anal. calc. for $C_{12}H_{11}N_3O_3$ (245.23): C 58.77, H 4.52, N 17.13; found: C 58.71, H 4.49, N 17.17.

*5,6-Dihydro-2-{4-[[(4-methylphenyl)sulfonyl]oxy}phenyl]-5-oxo-4*H-*1,3,4-oxadiazine-4-propanenitrile* (**27**): Yield 66%. M.p. 109°. IR (KBr): 2320, 1660. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.42 (*s*, 3 H); 2.92 (*t*, 2 H); 3.90 (*t*, 2 H); 4.85 (*s*, 2 H); 7.15–7.82 (*m*, 8 arom. H). Anal. calc. for $C_{19}H_{17}N_3O_5S$ (399.33): C 57.17, H 4.29, N 10.52; found: C 57.10, H 4.19, N 10.57.

*2-[4-(Benzoyloxy)phenyl]-5,6-dihydro-5-oxo-4*H-*1,3,4-oxadiazine-4-propanenitrile* (**28**): Yield 68%. M.p. 153°. IR (KBr): 2240, 1680. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.90 (*t*, 2 H); 3.91 (*t*, 2 H); 4.99 (*s*, 2 H); 6.81–7.42 (*m*, 9 arom. H). Anal. calc. for $C_{19}H_{15}N_3O_4$ (349.34): C 65.32, H 4.33, N 12.03; found: C 65.27, H 4.26, N 12.00.

*2-[4-(Benzyloxy)phenyl]-5,6-dihydro-5-oxo-4*H-*1,3,4-oxadiazine-4-propanenitrile* (**29**): Yield 76%. M.p. 140°. IR (KBr): 2240, 1680. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.90 (*t*, 2 H); 3.91 (*t*, 2 H); 4.84 (*s*, 2 H); 5.22 (*s*, 2 H); 7.20–7.35 (*m*, 9 arom. H). Anal. calc. for $C_{19}H_{17}N_3O_3$ (335.36): C 68.05, H 5.11, N 12.53; found: C 68.00, H 5.15, N 12.48.

*5,6-Dihydro-2-{3-[[(4-methylphenyl)sulfonyl]oxy}phenyl]-5-oxo-4*H-*1,3,4-oxadiazine-4-propanenitrile* (**30**): Yield 60%. M.p. 277°. IR (KBr): 2260, 1690. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.39 (*s*, 3 H); 2.82 (*t*, 2 H); 4.12 (*t*, 2 H); 4.78 (*s*, 2 H); 7.05–7.45 (*m*, 8 arom. H). Anal. calc. for $C_{19}H_{17}N_3O_5S$ (399.33): C 57.18, H 4.29, N 10.52; found: C 57.14, H 4.30, N 10.48.

*2-[3-(Benzoyloxy)phenyl]-5,6-dihydro-5-oxo-4*H-*1,3,4-oxadiazine-4-propanenitrile* (**31**). Yield 65%. M.p. 228°. IR (KBr): 2260, 1698, 1665. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.82 (*t*, 2 H); 3.95 (*t*, 2 H); 4.85 (*s*, 2 H); 6.90–7.91 (*m*, 9 arom. H). Anal. calc. for $C_{19}H_{15}N_3O_4$ (349.34): C 65.32, H 4.33, N 12.03; found: C 65.27, H 4.35, N 12.00.

*2-[3-(Benzyloxy)phenyl]-5,6-dihydro-5-oxo-4*H-*1,3,4-oxadiazine-4-propanenitrile* (**32**): Yield 63%. M.p. 95°. IR (KBr): 2100, 1660. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.85 (*t*, 2 H); 3.91 (*t*, 2 H); 4.85 (*s*, 2 H); 5.16 (*s*, 2 H); 7.10–7.45 (*m*, 9 arom. H). Anal. calc. for $C_{19}H_{17}N_3O_3$ (335.36): C 68.05, H 5.11, N 12.53; found: C 68.08, H 5.07, N 12.47.

*2-(1*H-*Indol-3-ylmethyl)-5,6-dihydro-5-oxo-4*H-*1,3,4-oxadiazine-4-propanenitrile* (**33**): Yield 85%. M.p. 89°. IR (KBr): 2350, 1685. ^{1}H-NMR (300 MHz, (D_6)DMSO): 2.82 (*t*, 2 H); 3.82 (*t*, 2 H); 4.40–4.70 (*m*, 4 H); 6.90–7.65 (*m*, 5 arom. H). Anal. calc. for $C_{15}H_{14}N_4O_2$ (282.30): C 63.82, H 5.00, N 19.85; found: C 63.76, H 4.93, N 19.88.

REFERENCES

[1] R. B. Hargreaves, B. J. McLoughlin, S. D. Mills, Eur. Pat. 85,227, 1983; *Chem. Abstr.* **1984**, *100*, 6561.

[2] I. Sircar, B. L. Duell, G. Bobowski, J. A. Bristol, D. B. Evans, *J. Med. Chem.* **1985**, *28*, 1405; W. H. Moos, C. C. Humblet, I. Sircar, C. Rithner, R. E. Weishaar, J. A. Bristol, A. T. McPhail, *J. Med. Chem.* **1987**, *30*, 1963; J. A. Bristol, I. Sircar, W. H. Moos, D. B. Evan, R. E. Weishaar, *J. Med. Chem.* **1984**, *27*, 1101; A. Mertens, W.-G. Friebe, B. Müller-Beckmann, W. Kampe, L. Kling, W. von der Saal, *J. Med. Chem.* **1990**, *33*, 2870.

[3] W. John, Eur. Pat. Appl. EP 220,044, 1987; *Chem. Abstr.* **1988**, *108*, 112503.

[4] K. Suhasini, R. T. V. Padmanabha, V. Thirupathaiah, *Curr. Sci.* **1983**, *52*, 1133; *Chem. Abstr.* **1984**, *100*, 156574.

[5] V. A. Budagov, S. D. Kyazimova, *Temat. Sb. Nauch. Tr. Azerb. NII Zashchity Rast.* **1979**, 79; *Chem. Abstr.* **1980**, *94*, 192289.

[6] M. A. Dekeyser, A. Mishra, R. C. Moore, *Chem. Abstr.* **1987**, *107*, 59057.

[7] M. A. Dekeyser, D. M. Borth, R. C. Moore, A. Mishra, *Chem. Abstr.* **1991**, *114*, 76982.

[8] K. D. Wing, M. E. Schnee, M. Sacher, M. Connair, *Arch. Insect. Biochem. Physiol.* **1998**, *37*, 91; S. M. M. Ismail, R. A. Baines, R. G. H. Downer, M. A. Dekeyser, *Pestic. Sci.* **1996**, *46*, 163.

[9] J. Wouters, F. Ooms, S. Jegham, J. J. Koenig, P. George, F. Durant, *Eur. J. Med. Chem.* **1997**, *32*, 721; P. Dostert, E. M. O'Brien, K. F. Tipton, M. Meroni, P. Melloni, M. S. Benedetti, *Eur. J. Med. Chem.* **1992**, *27*, 45; F. Mazouz, S. Gueddari, C. Burstein, D. Mansy, R. Milcent, *J. Med. Chem.* **1993**, *36*, 1157.

[10] D. Barton, W. D. Olis, 'Comprehensive Organic Chemistry', Pergamon Press Inc., New York, 1979, Vol. 2, p. 1051.

[11] R. L. Hinman, M. C. Flores, *J. Org. Chem.* **1959**, *24*, 660.

[12] F. Radat, J. Berlin, O. Speux-Varoquax, S. Elatki, M. Ferreri, A. J. Puech, *Psychopharmacology (Berlin)* **1996**, *127*, 370; M. Da Parada, R. Kettler, H. H. Keller, W. P. Burkard, D. Muggli-Maniglio, W. Haefely, *J. Pharmacol. Exp. Ther.* **1989**, *248*, 400.

[13] C. R. Ashby Jr., L. A. Carr, C. Cook, M. M. Steptoe, D. D. Franks, *Neuropsychobiology* **1992**, *25*, 121.
[14] G. C. Guilbault, P. J. Brignac, M. Juneau, *Anal. Chem*. **1968**, *40*, 1256.
[15] A. Dar, S. Khatoon, *Pharmacol*. *Biochem. Behav*. **2000**, *65*, 1.
[16] O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem*. **1951**, *193*, 265.
[17] P. J. R. Cannell, J. S. Kellam, M. A. Owsianka, M. J. Walker, *Planta Med*. **1988**, *54*, 10.
[18] R. L. Stein, A. M. Strimpler, *Biochemistry* **1987**, *26*, 2611.
[19] R. A. Carron, J. M. Maran, L. Montero, Fernandozalgo, A. A. Dominguez, *Plantes Medicinales et Phytother.* **1987**, *21*, 195; Atta-ur-Rahman, M. I. Choudhary, W. J. Thomsen, 'Bioassay Techniques for Drug Development', Harward Academic Press, Amsterdam, 2001, p. 16.
[20] H. Blank, G. Rewbell, *Arch. Derm*. **1965**, *92*, 322; S. S. Shaukat, N. A. Khan, F. Ahmed, *Pak. J. Bot*. **1980**, *12*, 97.

Received July 26, 2001