A Novel Series of 3,4-Disubstituted Dihydropyrazoles: Synthesis and Evaluation for MAO Enzyme Inhibition

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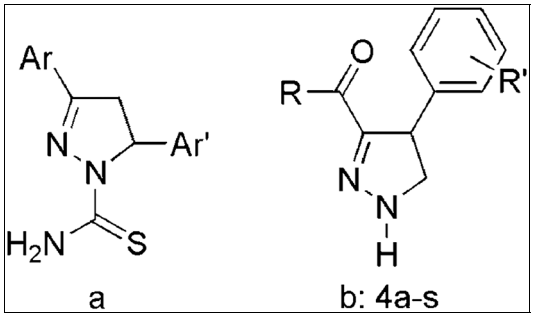

In this study, the authors have designed and synthesized a novel series of 3-acyl-4-aryl-4,5-dihydropyrazoles, with the aim to obtain new potential scaffolds for the inhibition of both isoforms of monoamine oxidase (MAO) enzyme. The synthetic pathway to these compounds includes as a key step the 1,3-dipolar cycloaddition reaction of diazomethane with a chalcone. All the compounds were fully characterized by means of spectroscopic and analytical data and showed specific inhibition against MAO A.

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INTRODUCTION

Dihydropyrazoles represent an important class of bioactive nitrogen heterocyclic compounds [1–18]. In particular, some dihydropyrazoles have played a crucial role in the development of heterocyclic chemistry and were also extensively used as key synthones in organic synthesis [19–22]. As a consequence, a large number of different substituted 2-pyrazoline derivatives were prepared [23–34].

A classical pathway for the synthesis of 1,3,5-trisubstituted dihydropyrazole is based on the reaction of compounds having α,β-unsaturated group in conjugation with carbonyl system with hydrazines.

Using this method, hydrazones were formed as intermediates, which can be subsequently cyclized to dihydropyrazole in the presence of a suitable cyclizing reagent like acetic acid.

The synthetic pathway to dihydropyrazoles by reaction of diazomethane with α,β-unsaturated ketones was first published by Azzarello [35], but he could not establish the exact position of the acyl moiety (either in the position 3 or 5 of the heterocyclic ring).

Later, this reaction was further investigated by a large number of researchers, whose published results were not always in agreement.

In fact, in the 1,3-dipolar cycloadditions of chalcones and diazomethane, the methylene part of the diazomethane could theoretically attack both the α and the β carbon atom.

The regioselectivity and the unequivocal structure of the resulting dihydropyrazole was later fully demonstrated by L. I. Smith [24,36] and mainly by Lévai *et al*. [37–41], who extended the characterization also to other polyunsaturated systems, using combined spectroscopic techniques (UV, IR, ^{1}H NMR, and ^{13}C NMR). The latter, further corroborate the regioselectivity of this reaction by thermal denitrogenation of the prepared dihydropyrazoles: the obtained 1,3-diaryl-3-methyl-propenone confirmed the attack of the methylene group of the diazomethane to the β-carbon atom of the α,β-enone during the cycloaddition reaction.

Despite the fact that several dihydropyrazole derivatives were found to possess important bioactivities, their activity as monoamine oxidase inhibiting agents (MAOI) has been reported in recent years only.

The research in this field has been developed after the discovery by Johnston [42] of two types of MAO, named MAO A and MAO B, and above all, the determination of the crystalline structure of human MAO B, registered in

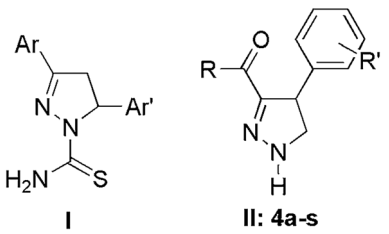


Figure 1. I: Structures of the previously published derivatives, active as MAOI; **Ar**: *p*-CH_3—C_6H_4, *p*-F—C_6H_4, thiophene; **Ar′**: *p*-CH_3—C_6H_4, *p*-Cl—C_6H_4, furan, thiophene. **II**: Dihydropyrazoles derivatives **4a–s**.

the RCSB Protein Data Bank [43], allowed the rational design of selective and reversible inhibitors of the enzyme. Both MAO isoforms are important in the metabolism of monoamine neurotransmitters and, as a result, MAO inhibitors (MAOIs) are studied for the treatment of several psychiatric and neurological disorders. In particular, MAO B inhibitors are adjuvant in the treatment of Parkinson's disease (PD) [44], while MAO A inhibitors are used as antidepressant and anxiolytic drugs. Furthermore, the activity of MAO B is enhanced by aging and in Alzheimer's disease (AD) patients [45]. Neurodegenerative disorders are associated with an increased MAO B activity. On the contrary, MAO A does not increase with age, suggesting that a totally independent mechanism regulates the expression of the two enzymatic isoforms. Therefore, new and wider applications for selective MAOIs are predictable.

In a previous study, we synthesized and studied some differently substituted 3,5-diaryl-4,5-dihydro-1-thiocarbamoylpyrazole derivatives [12] (Fig. 1, series I). Most of them showed very good inhibitory activities against MAO A and MAO B, some of them showed a very good reversible and selective activity against the B isoform. The compound of the series, which showed the best activity was again tested after the resolution of the racemic sample and its selectivity for MAO B increased twice and half for the (S)-(−)-enantiomer. On the basis of their activity and favorable therapeutic index, the dihydropyrazole derivatives have demonstrated to be good candidates for the inhibition of this enzyme.

RESULTS AND DISCUSSION

To further investigate the dihydropyrazole scaffold, the authors have now synthesized a new series of 3-acyl-4-aryl-4,5-dihydropyrazoles **4a–s** (Fig. 1, series II; Table 1).

All the newly synthesized compounds are characterized by a dihydropyrazole ring bearing either two aromatic substituents or a methyl aryl di-substitution (**4a**), which are differently bonded to the heterocyclic moiety, if compared with the previously synthesized compounds: compounds **4a–s** are substituted in the position 3 and 4 of the heterocyclic ring instead of position 3 and 5. It can be also noted that the chiral center is now present on C^4 instead of C^5. Moreover, there is a carbonyl spacer between the methyl or the aryl group in position 3 and the heterocyclic *core* and the N^1 of the dihydropyrazole is unsubstituted.

With respect to the previously reported compounds (**I**) the authors have introduced new aromatic substituents as well as already studied moieties.

The synthetic pathway for the preparation of **4a–s** compounds started from the reaction of an appropriately substituted enone with diazomethane. The appropriate chalcones **3a–s** were prepared slightly modifying the published report methods [46–48] by reacting equimolar amounts of the appropriate aldehyde and ketone in basic medium.

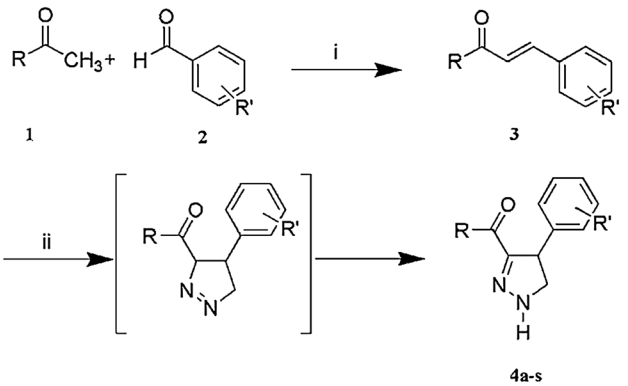
The diazomethane has been prepared following published report methods, starting from *N*-methyl-*N*-nitroso-*p*-toluensulfonamide (Diazald) or *N*-nitrosomethylurea [49, 50].

The 1,3-dipolar cycloaddition starts with the attachment of the diazomethane to the β-carbon of the chalcone, and the subsequent formation of the 1-pyrazoline. Afterward, rising to room temperature and during purification steps, 4,5-dihydro-3*H*-pyrazoles spontaneously rearrange to the themodynamically more stable 4,5-1*H*-dihydropyrazoles (Scheme 1). This synthetic procedure has been selected because it univocally leads to the desired regioisomer. In fact, the new dihydropyrazoles (**II**; Fig. 1) differ from the previously reported compounds (**I**; Fig. 1), only for the reciprocal position of the substituents and for the absence of the thiocarbamoyl group.

The compounds structures were fully characterized by means of mass spectrometry, elemental analyses, and spectroscopic techniques.

In fact, to further confirm the assigned structures, the authors perform ^{1}H NMR, ^{13}C NMR, and COSY spectra of compounds **4b** and **4i.** Protons on position 4 and 5 of the dihydropyrazole ring give rise to three sets of signals, as the authors could expect for a ABX system, but the

Scheme 1. Synthetic pathway to dihydropyrazole compounds **4a–s.**



Reagents and conditions. i: EtOH/ NaOH; ii: CH_2N_2, Et_2O, -10°C (1h), then r.t. (24h).

multiplicity of the signal ranging around 4 ppm, a triplet, is apparently not in agreement with the assigned structure, because the expected multiplicity was a doublet of doublets.

The partial decoupled ^{13}C NMR revealed one doublet at 47 ppm and one triplet at 58 ppm, which are due to the coupling of the C^4H and C^5H carbons with the bonded protons of the dihydropyrazole ring, respectively. The COSY spectra revealed two spin systems, one in the aromatic region and the other one in the region ranging between 3.5 and 4.8 ppm, which was composed by three sets of strictly coupled system, due to the ABX system, thus confirming the assigned structure. The authors were also able to confirm that the triplet ranging around 4 ppm effectively corresponds to the H_B proton of the ABX system and this unusual multiplicity is probably due to the similarity of the geminal (J_{AB}) with the cis (J_{BX}) coupling constants.

All compounds were tested to evaluate their capability to inhibit the two MAO isoforms. Compound **4k** showed the highest activity toward the A isoform of the enzyme (IC50 MAO A = 49.04 ± 2.53 μM). All the other compounds did not exhibit inhibitory activity at concentration lower than 100 μM. In general, it has been observed that both the introduction of the carbonyl spacer and the change in the position of the aryl moiety from C^5 to C^4, leads to a decrease of the enzymatic activity, with respect to the previously reported compounds.

Significantly all compounds are exclusively active, even though at relatively high concentration, on the MAO A isoform, suggesting that this structural modification could lead to a valid scaffold for the selective inhibition of the A isoform. These results are very encouraging. In fact, MAOAIs are capable to raise multiple monoamine levels with respect to other antidepressant agents such as selective serotonin reuptake inhibitors (SSRIs). In spite of this evident advantage, there is less development of MAOIs when compared with SSRIs. Therefore, the development of new, more selective and reversible MAOAIs is an attractive target. Prompted by these results, the authors will further investigate these derivatives, with the aim to obtain new and useful information for the design of more efficient molecules for the selective inhibition of the A isoform of MAO.

EXPERIMENTAL

Chemistry. Melting points are uncorrected and recorded on Electrothermal 9100 apparatus. Electron ionization mass spectra were obtained by a Fisons QMD 1000 mass spectrometer (70 eV, 200 μA, ion source temperature 200°C). Samples were directly introduced into the ion source. Found mass values are in agreement with theoretical ones. ^1H NMR spectra were performed on a Varian Unity 300 MHz, using deuterochloroform or dimethylsulfoxyde-d_6 as solvents and tetramethylsilane as internal standard. ^{13}C NMR and COSY spectra were performed on a Varian 400 MHz spectrometer. The chemical shifts (δ) were recorded in ppm units, and J are given in Hertz. The ^1H NMR splitting patterns are as follows: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; "t," false triplet; m, multiplet; br s, broad singlet. Microanalysis for CHN were carried out on a Carlo Erba 1106 analyzer.

The reactions were followed by tlc, using DC Alufolien Kieselgel 60 F 254 Merck plates.

All reagents and solvents were purchased from Sigma Aldrich, and used directly without further purifications.

General method for the synthesis of 3-acyl-4-aryl-4,5-dihydropyrazoles (4a–s). In a 500 mL flask under nitrogen flux were introduced 50 mL of an ethereal solution of diazomethane (16 mmol) and 100 mL of dry ethyl ether, maintained in an ethanol bath at −10°C by a cryostat. To this solution, a 150 mL ethereal solution of the appropriate chalcone (8 mmol) was added. The reaction mixture was stirred vigorously at −10°C for 1 h, then it was allowed to reach room temperature and further stirred for 24 h. The reaction was followed by tlc. At the end the solvent was removed under vacuo and a product was obtained, which was crystallized from isopropyl ether/methanol, ethanol.

3-Acetyl-4-(3,4-methylendioxyphenyl)-4,5-dihydropyrazole (4a). Bright yellow-colored product; *m/z* 232. ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 3.62 (dd, 1H, CH_2, J = 10.3, 4.8), 3.94 ("t," 1H, CH_2, J = 11.5, 10.3), 4.27 (dd, 1H, CH, J = 11.5, 4.8), 5.91 (s, 2H, O-CH_2-O), 6.30 (br s, 1H, NH, deuterium oxide exchangeable), 6.67 (d, 1H, phenyl proton, J = 8.8), 6.69 (s, 1H, phenyl proton), 6.71 (d, 1H, phenyl proton, J = 8.8). Anal. Calcd. for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06. Found: C, 61.81; H, 5.19; N, 12.01.

3-(4-Methylbenzoyl)-4-(3,4-methylendioxyphenyl)-4,5-dihydropyrazole (4b). Yellow powder; *m/z* 308. ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 3.64 (dd, 1H, CH_2, J = 10.3, 5.2), 4.01 ("t," 1H, CH_2, J = 12.0, 10.3), 4.53 (dd, 1H, CH, J = 12.2, 5.2), 5.88 (s, 2H, O-CH_2-O), 6.35 (br s, 1H, NH, deuterium oxide exchangeable), 6.70 (d, 1H, phenyl proton, J = 8.0), 6.76 (dd, 1H, phenyl proton, J =8.0, 1.7), 6.77 (d, 1H, phenyl proton, J = 1.7), 7.23 (d, 2H, phenyl protons, J = 8.0), 7.99 (d, 2H, phenyl protons, J = 8.0). ^{13}C NMR ($CDCl_3$): δ 186.8 (C=O), 153.0 (—C=N—), 148.0 (—C—O), 146.6 (—C—O), 140.3 (—C=), 135.0 (—C=), 134.7 (—C=), 130.3 (—C=), 128.7 (—C=), 120.4 (—C=), 108.5 (—C=), 107.6 (—C=), 101.0 (CH_2), 57.4 (—C—N), 48.6 (—C—), 21.6 (—CH_3). Anal. Calcd. for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.08. Found: C, 69.86; H, 5.26; N, 9.13.

3-(4-Fluorobenzoyl)-4-(3,4-dimethoxyphenyl)-4,5-dihydropyrazole (4c). Yellow crystals; *m/z* 328. ^1H NMR ($CDCl_3$): δ 3.70 (dd, 1H, CH_2, J = 10.3, 5.0), 3.90 (s, 6H, OCH_3), 4.03 ("t," 1H, CH_2, J = 12.0, 10.3), 4.60 (dd, 1H, CH, J = 12.0, 5.0), 6.43 (br s, 1H, NH, deuterium oxide exchangeable), 6.80 (d, 1H, phenyl proton, J = 8.4), 6.83 (d, 1H, phenyl proton, J = 8.4), 6.86 (s, 1H, phenyl proton), 7.12 (t, 2H, phenyl protons, J = 8.4), 8.18 (dd, 2H, phenyl protons, J = 8.4, 5.5). Anal. Calcd. for $C_{18}H_{17}FN_2O_3$: C, 65.84; H, 5.22; N, 8.53. Found: C, 66.05; H, 5.20; N, 8.49.

3-Benzoyl-4-(3,4-methylendioxyphenyl)-4,5-dihydropyrazole (4d). Yellow crystals; *m/z* 294. ^1H NMR ($CDCl_3$): δ 3.70 (dd, 1H, CH_2, J = 10.5, 5.0), 4.03 ("t," 1H, CH_2, J = 11.6, 10.5), 4.58 (dd, 1H, CH, J = 11.6, 5.0), 5.94 (s, 2H, O—CH_2—O), 6.44 (br s, 1H, NH, deuterium oxide exchangeable), 6.75 (d, 1H, phenyl proton, J = 8.4), 6.78 (d, 1H, phenyl proton, J = 8.4), 6.82 (d, 1H, phenyl proton, J = 1.7), 7.45 (t, 2H, phenyl protons, J = 7.8), 7.55

(t, 1H, phenyl proton, J = 7.8), 8.11 (d, 2H, phenyl protons, J = 7.9). Anal. Calcd. for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.17; H, 4.74; N, 9.47.

3-Benzoyl-4-(3,4-dichlorophenyl)-4,5-dihydropyrazole (4e). White product; *m/z* 318–320. ^{1}H NMR ($CDCl_3$): δ 3.71 (dd, 1H, CH_2, J = 10.5, 5.0), 4.05 ("t," 1H, CH_2, J = 11.9, 10.5), 4.58 (dd, 1H, CH, J = 11.9, 5.0), 6.58 (br s, 1H, NH, deuterium oxide exchangeable), 7.17 (dd, 1H, phenyl proton, J = 8.4, 2.1), 7.38 (d, 1H, phenyl proton, J = 8.4), 7.43 (t, 2H, phenyl protons, J = 7.6, 7.1), 7.48 (s, 1H, phenyl proton), 7.53 (t, 1H, phenyl proton, J = 7.1), 8.12 (d, 2H, phenyl protons, J = 7.6). Anal. Calcd. for $C_{16}H_{12}Cl_2N_2O$: C, 60.20; H, 3.79; N, 8.78. Found: C, 59.95; H, 3.81; N, 8.82.

3-(2-Thiophenoyl)-4-(3,4-methylendioxyphenyl)-4,5-dihydropyrazole (4f). Pale yellow crystals; *m/z* 300. ^{1}H NMR ($CDCl_3$): δ 3.70 (dd, 1H, CH_2, J = 10.1, 5.0), 4.02 ("t," 1H, CH_2, J = 11.8, 10.1), 4.52 (dd, 1H, CH, J = 11.8, 5.0), 5.93 (s, 2H, O—CH_2—O), 6.47 (br s, 1H, NH, deuterium oxide exchangeable), 6.75 (d, 1H, phenyl proton, J = 8.4), 6.78 (d, 1H, phenyl proton, J = 8.4), 6.79 (s, 1H, phenyl proton), 7.13 (t, 1H, thiophenyl proton, J = 3.8, 5.0), 7.63 (d, 1H, thiophenyl proton, J = 5.0), 8.21 (d, 1H, thiophenyl proton, J = 3.8). Anal. Calcd. for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 60.23; H, 4.01; N, 9.29.

3-(4-Methylbenzoyl)-4-(3,4-dimethoxyphenyl)-4,5-dihydropyrazole (4g). White crystals; *m/z* 324. ^{1}H NMR ($CDCl_3$): δ 2.44 (s, 3H, CH_3), 3.50 (dd, 1H, CH_2, J = 10.3, 5.0), 3.77 (s, 6H, OCH_3), 4.29 ("t," 1H, CH_2, J = 12.0, 10.3), 5.05 (dd, 1H, CH, J = 12.0, 5.0), 6.68 (br s, 1H, NH, deuterium oxide exchangeable), 6.71 (d, 1H, phenyl proton, J = 8.0), 6.73 (d, 1H, phenyl proton, J = 8.0), 6.75 (s, 1H, phenyl proton), 6.93 (d, 2H, phenyl protons, J_o = 8.0), 7.80 (d, 2H, phenyl protons, J_o = 8.0). Anal. Calcd. for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.61; H, 6.18; N, 8.60.

3-(4-Fluorobenzoyl)-4-(3,4-methylendioxyphenyl)-4,5-dihydropyrazole (4h). White crystals; *m/z* 312. ^{1}H NMR ($CDCl_3$): δ 3.70 (dd, 1H, CH_2, J = 10.3, 5.0), 4.03 ("t," 1H, CH_2, J = 12.0, 10.3), 4.56 (dd, 1H, CH, J = 12.0, 5.0), 5.94 (s, 2H, O—CH_2—O), 6.45 (br s, 1H, NH, deuterium oxide exchangeable), 6.75 (d, 1H, phenyl proton, J = 8.4), 6.77 (d, 1H, phenyl proton, J = 8.4), 6.79 (s, 1H, phenyl proton), 7.12 (t, 2H, phenyl protons, J = 8.8), 8.18 (dd, 2H, phenyl protons, J = 8.8, 5.7). Anal. Calcd. for $C_{17}H_{13}FN_2O_3$: C, 65.40; H, 4.20; N, 8.97. Found: C, 65.66; H, 4.08; N, 9.01.

3-(4-Trifluoromethylbenzoyl)-4-(3,4-methylendioxyphenyl)-4,5-dihydropyrazole (4i). Pale yellow crystals; *m/z* 362. ^{1}H NMR ($CDCl_3$): δ 3.70 (dd, 1H, CH_2, J = 10.5, 5.3), 4.03 ("t," 1H, CH_2, J = 12.1, 10.5), 4.52 (dd, 1H, CH, J = 12.1, 5.3), 5.90 (s, 2H, O—CH_2—O), 6.54 (s, 1H, NH, deuterium oxide exchangeable), 6.72 (d, 1H, phenyl proton, J = 8.4), 6.74 (d, 1H, phenyl proton, J = 8.4), 6.75 (d, 1H, phenyl proton, J =1.26), 7.65 (t, 2H, phenyl protons, J = 8.4), 8.15 (d, 2H, phenyl protons, J = 8.4). ^{13}C NMR ($CDCl_3$): δ 185.9 (C=O), 152.1 (—C=N—), 148.0 (—C—O), 146.7 (—C—O), 140.3 (—C=), 134.8 (—C=), 130.3 (—C=), 129.8 (—C=), 124.9 (—C=), 124.8 (CF_3), 120.3 (—C=), 108.5 (—C=), 107.5 (—C=), 101.0 (CH_2), 57.7 (—C—N), 48.0 (—C—). Anal. Calcd. for $C_{18}H_{13}F_3N_2O_3$: C, 59.67; H, 3.62; N, 7.73. Found: C, 59.93; H, 3.60; N, 7.69.

3-(2-Methylbenzoyl)-4-(4-chlorophenyl)-4,5-dihydropyrazole (4j). Yellow crystals; *m/z* 298–300. ^{1}H NMR (DMSO): δ 2.26 (s, 3H, CH_3), 3.74 (dd, 1H, CH_2, J = 10.4, 5.4), 4.10 (dd, 1H, CH_2, J = 12.0, 10.8), 4.58 (dd, 1H, CH, J = 12.0, 5.4), 6.42 (br s, 1H, NH, deuterium oxide exchangeable), 7.20 (d, 2H, phenyl protons, J = 7.5), 7.23-7.35 (m, 4H, phenyl protons), 7.44 (d, 2H, phenyl protons, J = 8.7). Anal. Calcd. for $C_{17}H_{15}ClN_2O$: C, 68.34; H, 5.06; N, 9.38. Found: C, 68.59; H, 5.08; N, 9.43.

3-(3-Methylbenzoyl)-4-(4-chlorophenyl)-4,5-dihydropyrazole (4k). Pale yellow crystals; *m/z* 298–300. ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H, CH_3), 3.71 (dd, 1H, CH_2, J = 10.5, 5.4), 4.07 (dd, 1H, CH_2, J = 11.4, 10.5), 4.61 (dd, 1H, CH, J = 11.7, 5.4), 6.45 (br s, 1H, NH, deuterium oxide exchangeable), 7.10–7.32 (m, 6H, phenyl protons), 7.87 (d, 2H, phenyl protons, J = 8.7). Anal. Calcd. for $C_{17}H_{15}ClN_2O$: C, 68.34; H, 5.06; N, 9.38. Found: C, 68.55; H, 5.04; N, 9.41.

3-(4-Methylbenzoyl)-4-(4-chlorophenyl)-4,5-dihydropyrazole (4l). Pale yellow crystals; *m/z* 298–300. ^{1}H NMR ($CDCl_3$): δ 2.39 (s, 3H, CH_3), 3.70 (dd, 1H, CH_2, J = 10.2, 5.1), 4.06 (dd, 1H, CH_2, J = 12.0, 10.2), 4.61 (dd, 1H, CH, J = 12.0, 5.1), 6.42 (br s, 1H, NH, deuterium oxide exchangeable), 7.21-7.29 (m, 7H, phenyl protons), 8.00 (d, 2H, phenyl protons, J = 8.4). Anal. Calcd. for $C_{17}H_{15}ClN_2O$: C, 68.34; H, 5.06; N, 9.38. C, 68.58; H, 5.03; N, 9.42.

3-(2,4-Dichlorobenzoyl)-4-(4-chlorophenyl)-4,5-dihydropyrazole (4m). Pale yellow crystals; *m/z* 351–353. ^{1}H NMR ($CDCl_3$): δ 3.78 (dd, 1H, CH_2, J = 10.6, 5.5), 4.17 (dd, 1H, CH_2, J = 12.1, 10.6), 4.57 (dd, 1H, CH, J = 12.1, 5.5), 6.62 (br s, 1H, NH, deuterium oxide exchangeable), 7.24–7.33 (m, 6H, phenyl protons), 7.42 (s, 1H, phenyl proton). Anal. Calcd. for $C_{16}H_{11}Cl_3N_2O$: C, 54.34; H, 3.14; N, 7.92. Found: C, 54.49; H, 3.15; N, 7.89.

3-(3-Methoxybenzoyl)-4-(4-chlorophenyl)-4,5-dihydropyrazole (4n). Pale yellow crystals; *m/z* 314–316. ^{1}H NMR ($CDCl_3$): δ 3.70 (dd, 1H, CH_2, J = 10.5, 5.4), 3.82 (s, 3H, OCH_3), 4.07 (dd, 1H, CH_2, J = 11.7, 10.5), 4.60 (dd, 1H, CH, J = 12.0, 5.1), 6.44 (br s, 1H, NH, deuterium oxide exchangeable), 7.05–7.35 (m, 6H, phenyl protons), 7.60 (dd, 1H, phenyl proton, J = 1.5, 2.7), 7.74 (dt, 1H, phenyl proton, J = 7.8, 1.5). Anal. Calcd. for $C_{17}H_{15}ClN_2O_2$: C, 64.87; H, 4.80; N, 8.90. Found: C, 64.99; H, 4.78; N, 8.87.

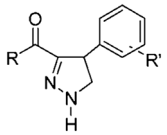
3-(4-Methoxybenzoyl)-4-(4-chlorophenyl)-4,5-dihydropyrazole (4o). Yellow crystals; *m/z* 314–316. ^{1}H NMR ($CDCl_3$): δ 3.67 (dd, 1H, CH_2, J = 10.5, 5.1), 3.85 (s, 3H, OCH_3), 4.03 (dd, 1H, CH_2, J = 11.7, 10.2), 4.61 (dd, 1H, CH, J = 11.7, 5.4), 6.37 (br s, 1H, NH, deuterium oxide exchangeable), 6.90 (d, 2H, phenyl protons, J = 9.0), 7.24 (s, 4H, phenyl protons), 8.16 (d, 2H, phenyl protons, J = 8.5), 8.07 (d, 2H, phenyl protons, J = 8.7). Anal. Calcd. for $C_{17}H_{15}ClN_2O_2$: C, 64.87; H, 4.80; N, 8.90. Found: C, 64.62; H, 4.81; N, 8.93.

3-(4-Chlorobenzoyl)-4-(4-chlorophenyl)-4,5-dihydropyrazole (4p). Pale yellow crystals; *m/z* 318–320. ^{1}H NMR ($CDCl_3$): δ 3.72 (dd, 1H, CH_2, J = 10.7, 5.2), 4.08 ("t," 1H, CH_2, J = 11.6, 10.7), 4.60 (dd, 1H, CH, J = 11.9, 5.2), 6.46 (br s, 1H, NH, deuterium oxide exchangeable), 7.23 (d, 2H, phenyl protons, J = 8.5), 7.27 (d, 2H, phenyl protons, J = 8.2), 7.38 (d, 2H, phenyl protons, J = 8.5), 8.07 (d, 2H, phenyl protons, J = 8.5). Anal. Calcd. for $C_{16}H_{12}Cl_2N_2O$: C, 60.21; H, 3.80; N, 8.77. Found: C, 59.98; H, 3.83; N, 8.80.

3-(3-Methylbenzoyl)-4-phenyl-4,5-dihydropyrazole (4q). Yellow powder, *m/z* 264. ^{1}H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3); 3.73 (dd, 1H, CH_2, J = 10.5, 5.4); 4.06 ("t," 1H, CH_2, J = 11.4, 10.5); 4.65 (dd, 1H, CH, J = 11.4, 5.1); 6.39 (br s, 1H, NH, deuterium oxide exchangeable); 7.20–7.31 (m, 7H, phenyl protons); 7.86–7.90 (m, 2H, phenyl protons). Anal. Calcd. for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60. Found: C, 77.02; H, 6.08; N, 10.55.

Table 1

Synthesized compounds **4a–s**.



Compound	R	R′	Yield (%)	M.P. (°C; cryst. solv.)
4a	CH_3	3,4—O—CH_2—O—	62	114–115 (Isoprop. ether/CH_3OH)
4b	4—CH_3—C_6H_4	3,4—O—CH_2—O—	75	139–140 (EtOH)
4c	4—F—C_6H_4	3,4—di—CH_3O	71	121–122 (EtOH)
4d	C_6H_5	3,4—O—CH_2—O—	77	164–167 (EtOH)
4e	C_6H_5	3,4—di—Cl	81	160–163 (EtOH)
4f	c—C_4H_3S	3,4—O—CH_2—O—	78	150–151 (EtOH)
4g	4—CH_3—C_6H_4	3,4—di—CH_3O	60	163–165 (EtOH)
4h	4—F—C_6H_4	3,4—O—CH_2—O—	75	170–172 (EtOH)
4i	4—CF_3—C_6H_4	3,4—O—CH_2—O—	66	150–151 (EtOH)
4j	2—CH_3—C_6H_4	4—Cl–	65	129–131 (EtOH)
4k	3—CH_3—C_6H_4	4—Cl–	70	132–134 (EtOH)
4l	4—CH_3—C_6H_4	4—Cl–	72	174–175 (EtOH)
4m	2,4—di—Cl—C_6H_3	4—Cl–	68	162–165 (EtOH)
4n	3—CH_3O—C_6H_4	4—Cl–	74	131–133 (EtOH)
4o	4—CH_3O—C_6H_4	4—Cl–	80	149–150 (EtOH)
4p	4—Cl—C_6H_4	4—Cl–	67	157–159 (EtOH)
4q	3—CH_3—C_6H_4	H	83	131–132 (EtOH)
4r	4—CH_3—C_6H_4	H	75	140–141 (EtOH)
4s	4—CH_3O—C_6H_4	H	73	145–146 (EtOH)

3-(4-Methylbenzoyl)-4-phenyl-4,5-dihydropyrazole (4r). Yellow powder; *m/z* 264. ^{1}H NMR (DMSO): δ 2.38 (s, 3H, CH_3), 3.72 (dd, 1H, CH_2, J = 10.3, 5.3), 4.06 (dd, 1H, CH_2, J = 11.5, 10.3), 4.65 (dd, 1H, CH, J = 11.7, 5.5), 6.36 (br s, 1H, NH, deuterium oxide exchangeable), 7.20–7.31 (m, 7H, phenyl protons), 8.02 (t, 2H, phenyl protons, J = 8.2). Anal. Calcd. for $C_{17}H_{16}N_2O$: C, 77.25; H, 6.10; N, 10.60. Found: C, 77.47; H, 6.07; N, 10.63.

3-(4-Methoxybenzoyl)-4-phenyl-4,5-dihydropyrazole (4s). White crystals; *m/z* 280. ^{1}H NMR ($CDCl_3$): δ 3.71 (dd, 1H, CH_2, J = 10.1, 5.5), 3.84 (s, 3H, OCH_3), 4.04 (dd, 1H, CH_2, J = 11.6, 10.3), 4.65 (dd, 1H, CH, J = 11.7, 5.5), 6.33 (br s, 1H, NH, deuterium oxide exchangeable), 6.90 (d, 2H, phenyl protons, J = 8.8), 7.24–7.30 (m, 5H, phenyl protons), 8.16 (d, 2H, phenyl protons, J = 8.6). Anal. Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.63; H, 5.77; N, 10.04.

Enzymatic activity. The potential effects of test compounds against hMAOs were investigated by measuring their effects on the production of H_2O_2 from *p*-tyramine, using the Amplex Red MAO assay kit, and following the general procedure previously described by some of the authors [51].

Briefly, microsomal MAO isoforms prepared from insect cells (BTI-TN-5B1-4) in adequate amounts to oxidize 165 pmol of *p*-tyramine/min were incubated for 15 min with the new compounds at 37°C in a flat-black-bottom 96-well microtest plate (BD Biosciences, Franklin Lakes, NJ). The reaction was started by adding 200 μM Amplex Red reagent, 1 U/mL horseradish peroxidase and 1 mM *p*-tyramine and the production of H_2O_2 and, consequently, of resorufin was quantified at 37°C in a multidetection microplate fluorescence reader (Fluostar Optima, BMG Labtech GmbH, Offenburg, Germany) based on the fluorescence generated (excitation 545 nm, emission 590 nm) over a 15-min period, in which, the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the test drugs (new compounds and reference inhibitors) with appropriate dilutions of the vehicles.

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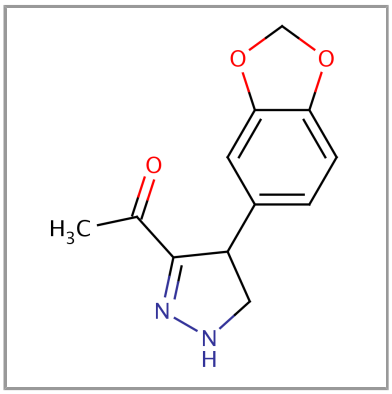

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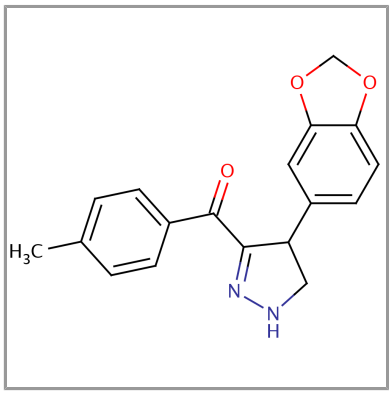

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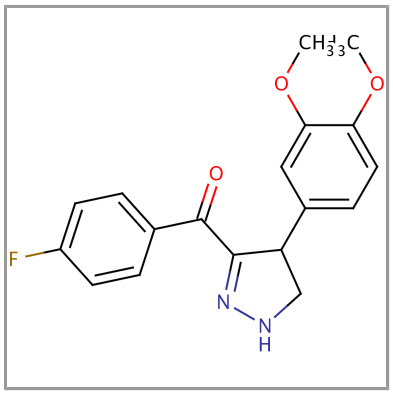

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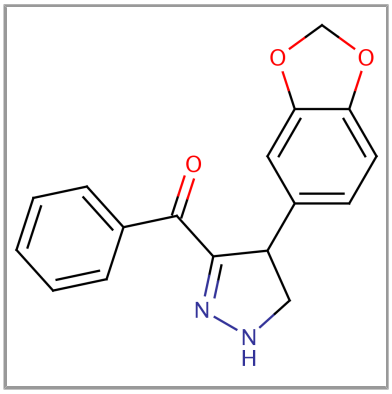

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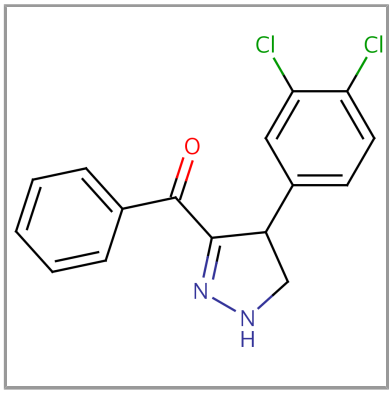

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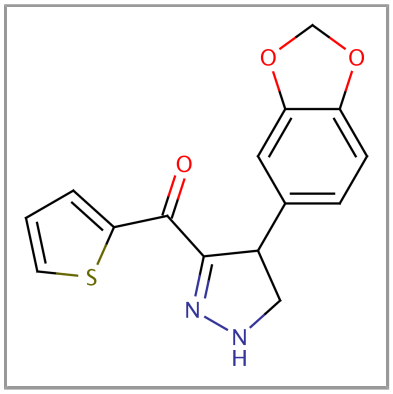

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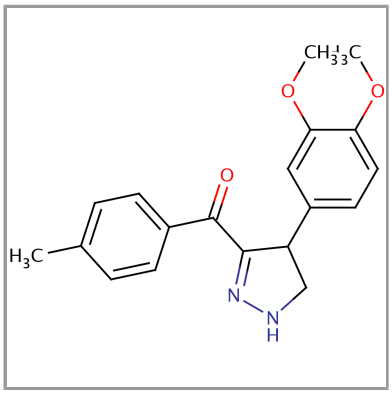

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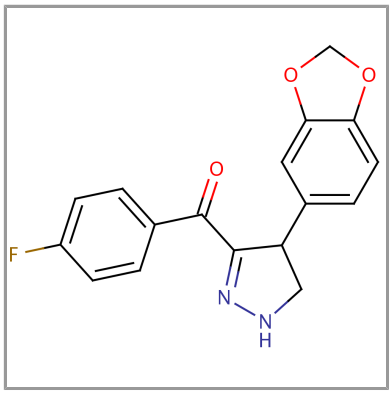

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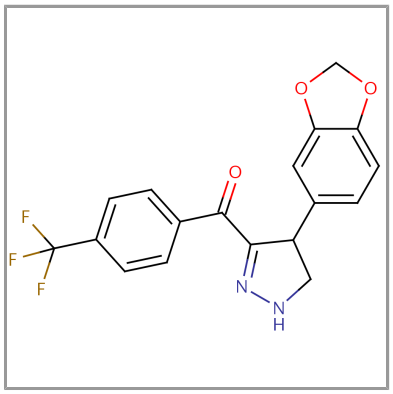

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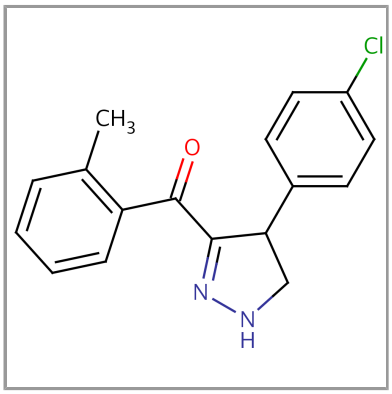

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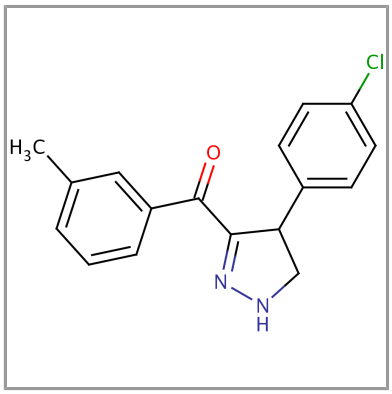

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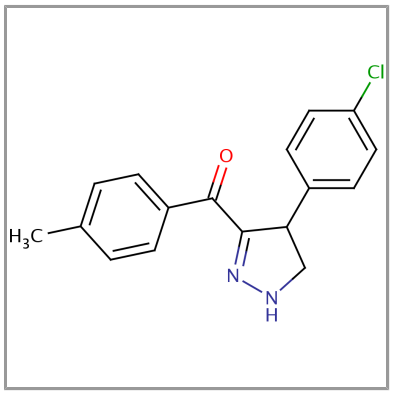

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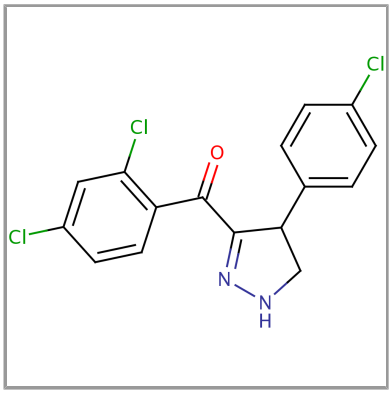

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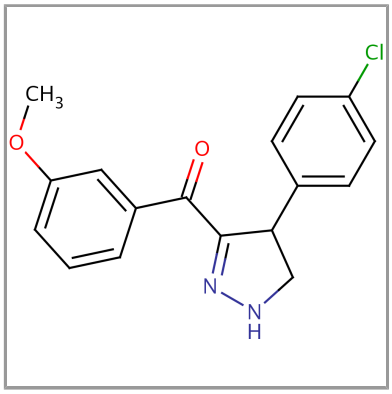

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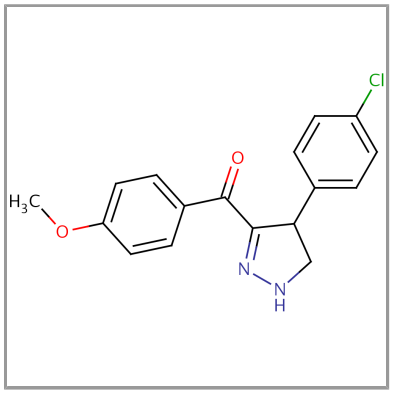

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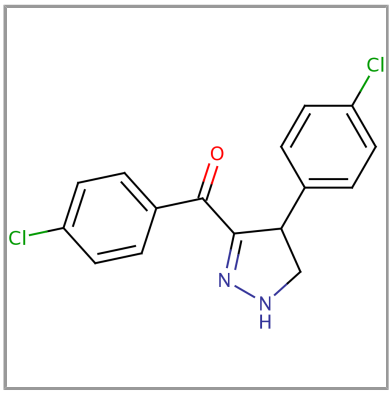

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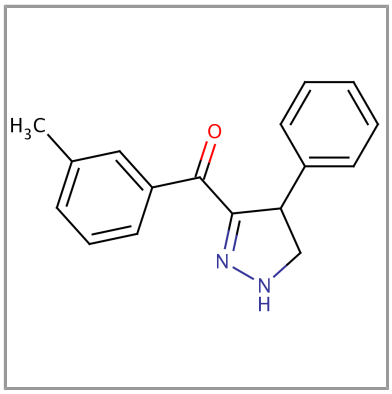

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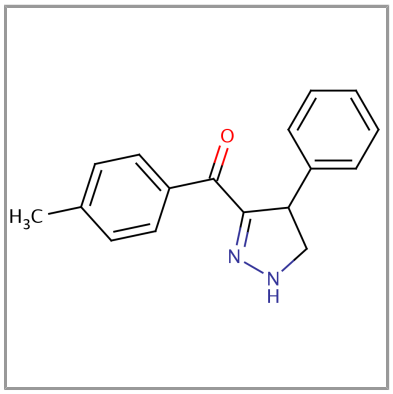

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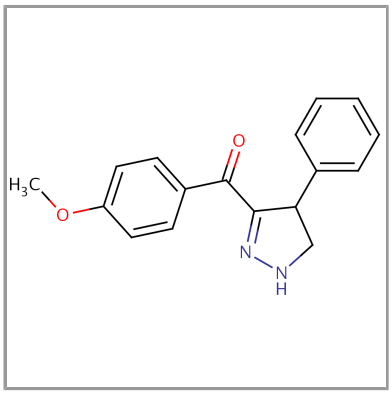
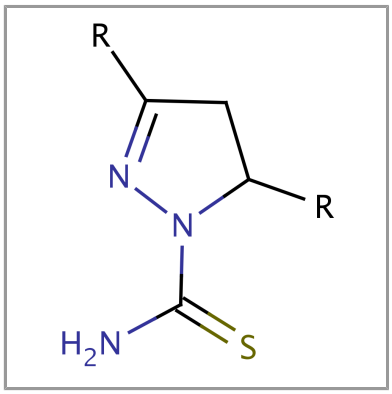
4s



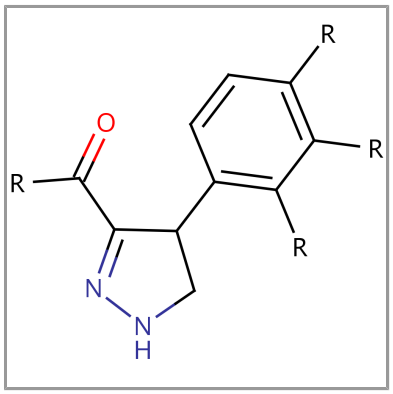
Compound Details Structure Search

I



Compound Details Structure Search

II



Compound Details Structure Search

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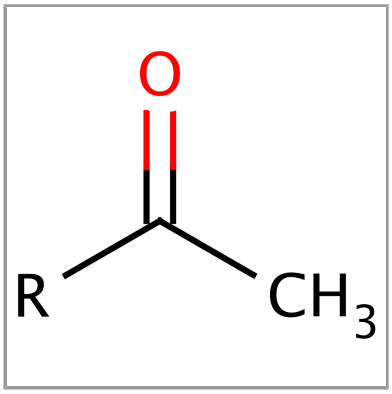

Compound Details Structure Search

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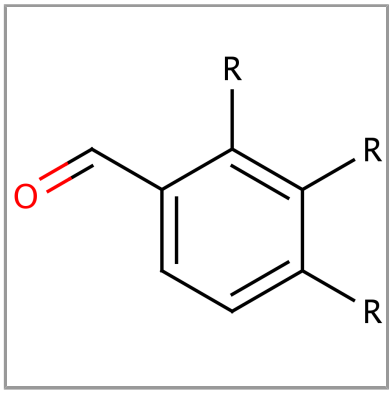

Compound Details Structure Search

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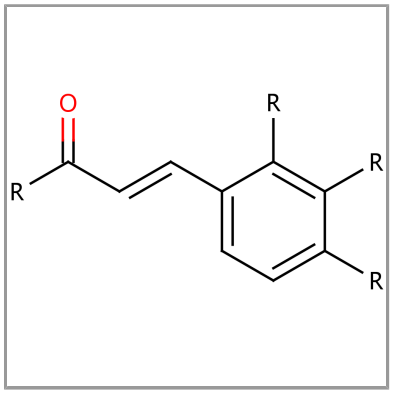

Compound Details Structure Search

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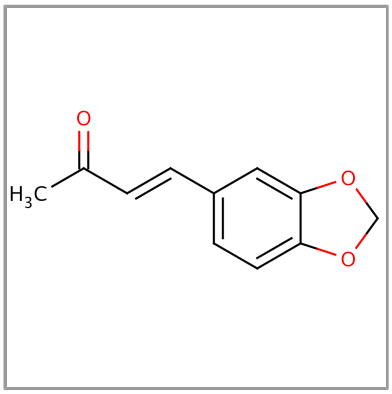

Compound Details Structure Search

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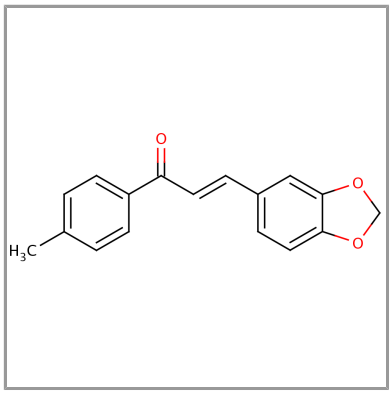

Compound Details Structure Search

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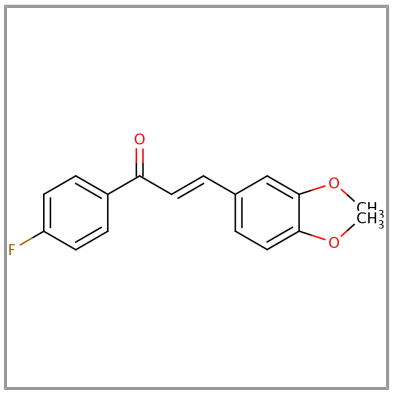

Compound Details Structure Search

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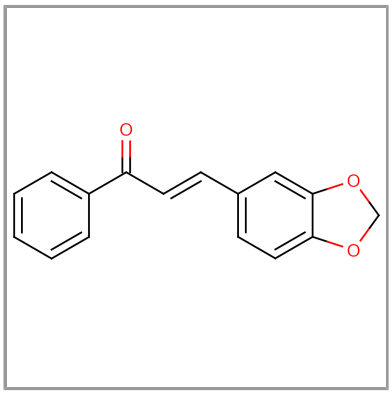

Compound Details Structure Search

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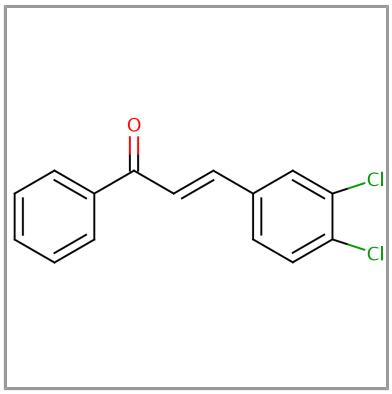
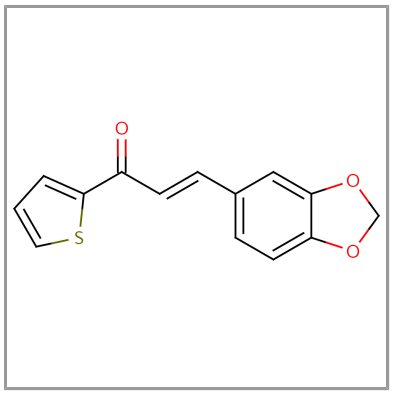


Compound Details Structure Search

3f



Compound Details Structure Search

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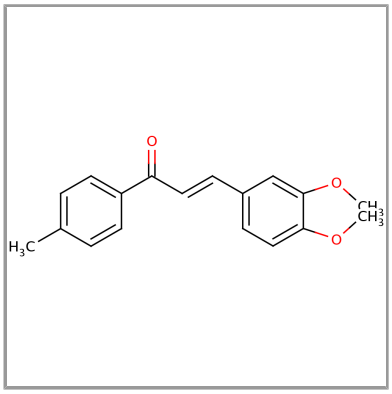
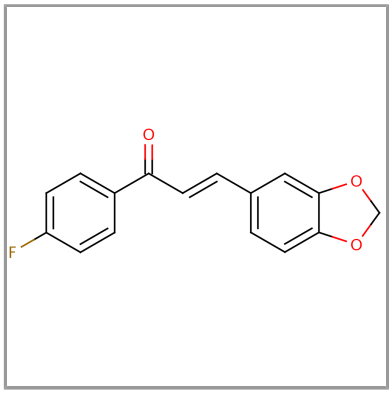


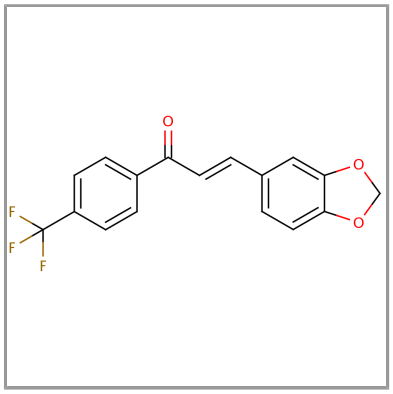
Compound Details Structure Search

3h



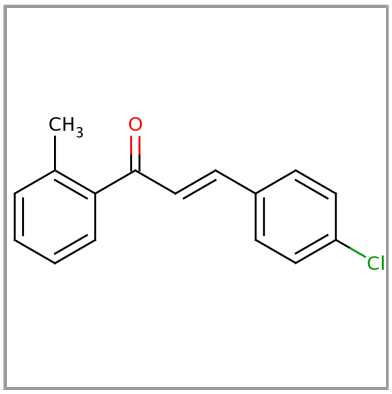
Compound Details Structure Search

3i



Compound Details Structure Search

3j



Compound Details Structure Search

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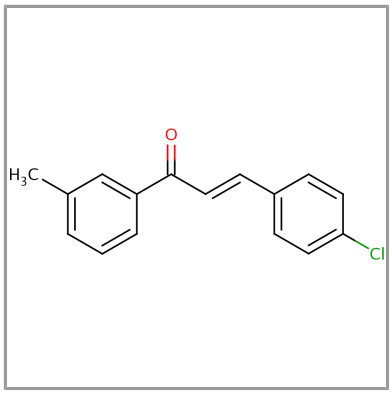

Compound Details Structure Search

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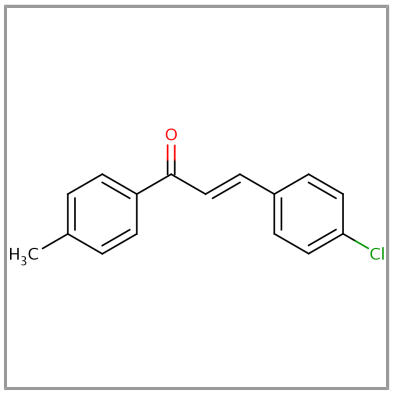

Compound Details Structure Search

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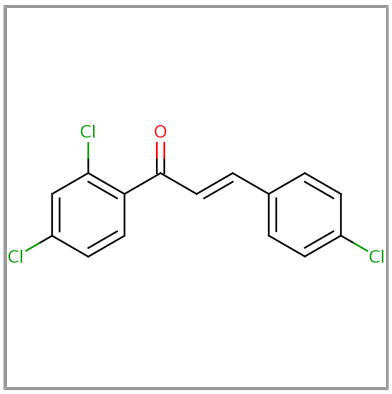

Compound Details Structure Search

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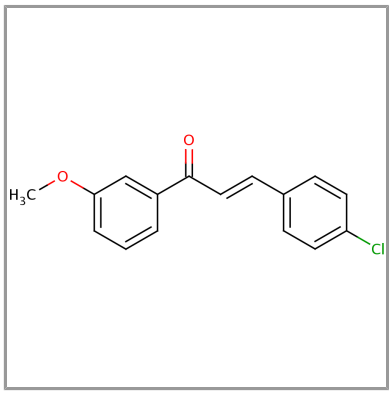

Compound Details Structure Search

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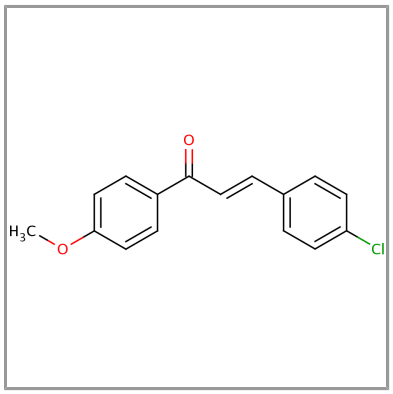
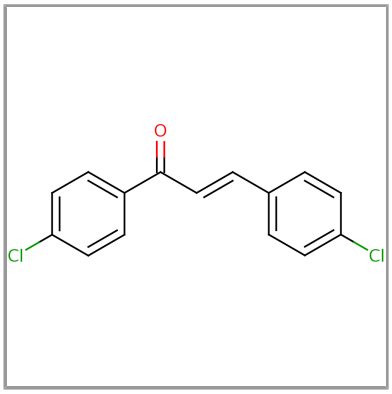


Compound Details Structure Search

3p



Compound Details Structure Search

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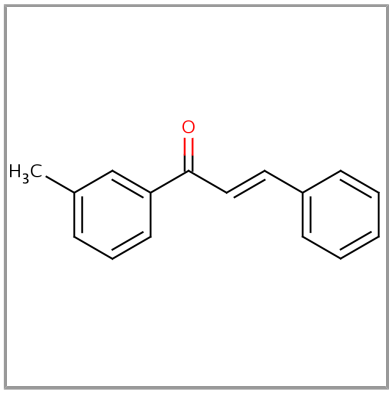
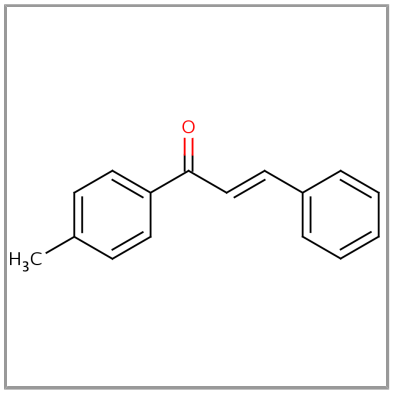


Compound Details Structure Search

3r



Compound Details Structure Search

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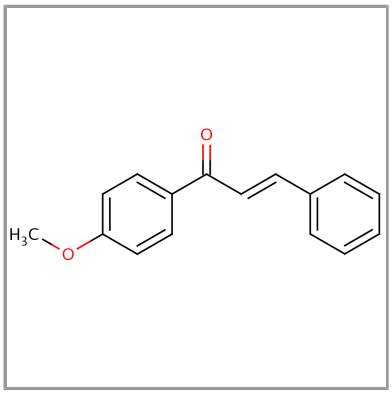
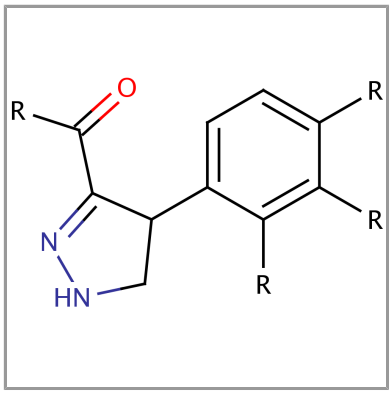


Compound Details Structure Search

4



Compound Details Structure Search