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# Synthesis, antihistaminic action and theoretical studies of (4-methoxybenzyl)(1,4,5,6-tetrahydropirimidin-2-yl) amine hydroiodide

**Murat Genc · Engin Yılmaz · Selcuk Ilhan · Zuhal Karagoz**

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**Abstract** In this study, (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl) amine hydroiodide (**2**) was synthesized by reaction of 2-methylmercapto-1,4,5, 6-tetrahydropyrimidine hydroiodide (**1**) and 4-methoxybenzylamine. The synthesized compound was tested for its in vitro H1-antihistaminic activity on guinea pig trachea. A promising bronchorelaxant effect of **2** was observed in histamine-contracted guinea pig tracheal chain via H1 receptor antagonism. In addition, the molecular geometry and gauge including atomic orbital (GIAO) $^{1}$H chemical shift values of the title compound in the ground state were calculated using the density functional method (DFT/UB3LYP) and Hartree–Fock (HF) approach using 6–311G+(d), 6–311G+(d,p), LANL2DZ, DGDZVP, and DGDZVP2 basis sets and compared with the experimental data. According to the experimental and theoretical results, HF/6–311G+(d) showed a better fit to experimental values in evaluating $^{1}$H-nuclear magnetic resonance (NMR) chemical shift values. Theoretical studies supported our findings, revealing the $N_{12}$ atom as the most nucleophilic. In addition, other structures of the compound such as the aromatic ring and $OCH_3$ group increased this property.

M. Genc (✉)
Department of Chemistry, Faculty of Science and Arts, Adiyaman University,
02040 Adiyaman, Turkey
e-mail: mgenc23@gmail.com

E. Yılmaz
Department of Chemistry, Faculty of Science and Art, University of Bitlis Eren,
13800 Bitlis, Turkey

S. Ilhan
Department of Pharmacology, Faculty of Medicine, University of Firat, 23119 Elazig, Turkey

Z. Karagoz
Department of Chemistry, Faculty of Science, University of Firat, 23119 Elazig, Turkey

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## Introduction

Pyrimidine and its derivatives belong to the family of nucleic acids. Pyrimidine-containing compounds have been of considerable interest in the field of medicinal chemistry, e.g., as inhibitors of human nitric oxide synthase isoforms, calcium channel blockers, antihypertensives, anti-inflammatory agents, analgesics, and $\alpha v\beta 3$ antagonists, and in the treatment of intestinal disorders, as well as demonstrating antibacterial, antifungal, and anti-human immunodeficiency virus (HIV) activities [1–8]. Specifically, drugs containing a pyrimidine with an oxygen in the 5-position have found a wide range of applications in several therapeutic areas. Hirabayashi et al. [9] found that imidazo[1,2-*c*]pyrimidine derivatives potently inhibit the Syk family of kinases. Also, pyridopyrimidines are bioactive nitrogen heterocyclic compounds, and several of their substituted derivatives have been associated with diverse immunopharmacological activities such as analgesic, anti-inflammatory, antiallergic, antiplatelet aggregatory, and antihistaminic actions [10–20].

The prevalence of asthma and other allergic diseases is increasing, providing a rapidly expanding market for antiallergic drugs. First-generation antihistamines penetrate the blood–brain barrier, and also possess anticholinergic properties, which led to the development of a second generation of H1-antagonists, such as terfenadine, cetirizine, and astemizole [21]. A common feature of first-generation compounds is two aryl or heteroaryl rings linked to an aliphatic tertiary amine via the side chain (e.g., diphenhydramine and pheniramine) [22], and second-generation compounds (terfenadine and cetirizine) also contain many of the structural features of first-generation compounds. The real breakthrough in nonsedative antihistamines came in the early 1980s when modern antihistamines were discovered, being found to exhibit potent antihistaminic activity without sleep-inducing effect [23]. This new generation of H1-antihistamines containing condensed heterocycles (e.g., loratadine, azelastine, and flazelastine) and lacking the above-mentioned pharmacophore led to the discovery of many novel antihistamines such as temelastine and mangostin [24, 25].

Prediction of the molecular and spectroscopic properties of molecules is an important part in the design process. One of the most widely used methods to calculate ground-state geometries in computational chemistry is density functional theory (DFT). It is known that the absorption spectrum is related to the molecular structure, and a relationship between the absorption maximum and the structure is much desired. To achieve this, the experimental spectrum can be compared with the calculated one. The extension of DFT to excited states is known as time-dependent density functional theory (TD-DFT) and has been used to describe the optical and spectroscopic properties of medium-sized and large molecules [26–30].

The localized Hartree–Fock (LHF) method, which is applied for the calculation of NMR shielding constants in this study, represents an efficient and effective, exact exchange method that is self-interaction free by construction, therefore leading to

improved orbital energy spectra [31, 32]. Moreover, unlike the parent Hartree–Fock method and hybrid functionals, it provides a local potential and thus allows for efficient calculation of NMR shielding constants without the need to solve the coupled Kohn–Sham equations if, as usual, current dependencies are neglected [33].

The antihistaminic action and theoretical calculations of 2-amino-1,4,5,6-tetrahydropyrimidine compounds have not been described in the literature to date. The aim of this study is to synthesize (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl)amine hydroiodide and investigate its quantum parameters and antihistaminic properties.

## Experimental

### Instrumentation and chemicals

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Histamine was purchased from Sigma Aldrich Inc. (St. Louis, MO, USA). Infrared (IR) spectrum was determined on a PerkinElmer Spectrum One Fourier-transform infrared (FT-IR) spectrometer. NMR spectra were recorded on a Bruker 300-MHz spectrometer. Melting point (uncorrected) was determined with the the Stuart SMP 30 apparatus.

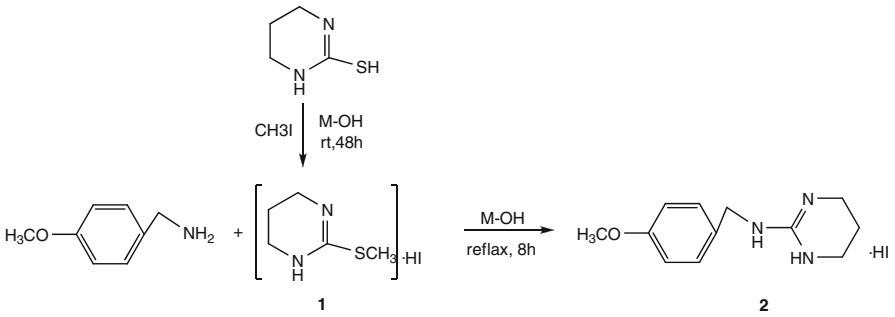
In this study, five guinea pigs were used. Guinea pigs (300–400 g) were procured from Firat University Experimental Research Institute. Animals were maintained under regulated environmental conditions (temperature $24 \pm 2$ °C, humidity 50–80 %, 12 h light/dark cycle) and provided with pelleted rodent feed and water ad libitum. The Institution of Firat university experimental research Animal Ethics Committee approved the animal experiments.

### Preparation of 2-methylmercapto-1,4,5,6-tetrahydropyrimidine hydroiodide

1,4,5,6-Tetrahydropyrimidine-2-thione (0.01 mol) and methyl iodide (0.01 mol) in 50 mL methanol were left at ambient temperature in a closed flask for 48 h. Then the mixture was heated under reflux for 6 h. The solvent was removed by evaporation; oily residue was cooled and triturated with diethyl ether. The crude product was collected, and washed with cold ethanol [34, 35].

### Synthesis of (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl)amine hydroiodide

A solution of 4-methoxybenzylamine (2 mmol) and 2-methylmercapto-1,4,5,6-tetrahydropyrimidine hydroiodide (1 mmol) in methanol (15 ml) were stirred at room temperature. The mixture was refluxed for 8 h. The mixture was then poured into diethyl ether, and the precipitate obtained was filtered, dried, and recrystallized from ethanol.




**Scheme 1** Synthesis of (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl)amine hydroiodide

*Caution*

Noxious gas $CH_3SH$ is evolved during the synthesis and should be trapped with a concentrated aqueous solution of NaOH and then destroyed with sodium hypochlorite.

Colorless; mp.: 150–153 °C; IR (near, $cm^{-1}$) $\nu_{max}$: 3150, 3004, 2998, 2909, 2986, 1638, 1610, 1254; $^1$H-NMR (300 MHz, $CHCl_3$-d): 1.95 (m, 2H, pyrimidine $CH_2$), 3.36 (m, 4H, pyrimidine N–$CH_2$), 3.82 (s, 3H, $OCH_3$), 4.38 (s, 2H, benzylic $CH_2$), 6.75 (br, 1H, NH), 6.91 (d, J: 8.4 Hz, 2H, Ar–H), 7.33 (d, J: 8.7 Hz, 2H, Ar–H), 7.49 (1H, $^+$NH). 8.00 (br, 1H, NH); $^{13}$C-NMR (75 MHz, $CHCl_3$-d): 159.89, 130.95, 129.25, 126.24, 114.48, 55.73, 55.67, 42.28, 38.60; Anal. calcd. for $C_{12}H_{18}IN_3O$ (347.20): C, 41.51; H, 5.23; N, 12.10;. Found: C, 41.59; H, 5.38; N, 12.17 (yield 57 %). (Scheme 1)

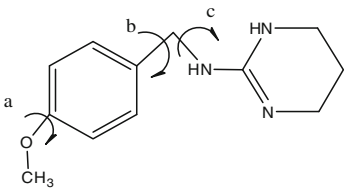
Determination of smooth muscle relaxant activity

Trachea (cleaned of adherent fat and connective tissue) was removed from guinea pigs and put into a Petri dish containing Krebs–Henseleit solution prewarmed to 37 °C. The tracheal chain was then mounted under initial tension of 2 g in the organ bath filled with Krebs–Henseleit solution maintained at 37 °C. The pH of the buffer was maintained at 7.4 by continuous aeration of the organ bath with 95 % $O_2$/5 % $CO_2$ mixture. The strip was allowed to equilibrate for 1 h and rinsed every 15 min with buffer. After the equilibration period, contraction was elicited by addition of histamine to the bath volume (submaximal effect). The maximum effect was observed with 1.25 mM histamine (cumulative). Pyrimidine solutions were added from $1 \times 10^{-9}$ to $1 \times 10^{-1}$ M. The mean values of relaxation were recorded.

Computational procedure

In the first step of the calculation, the potential energy surface (PES) of the molecule was obtained by performing a relaxed scan of dihedral angles using the semiempirical method at the PM3 level of theory. Geometrical parameters of this

structure were further optimized by using density functional theory and HF methods on the basis of the lowest energy conformer: the potential energy curve (PES) of molecule was obtained performing a relaxed scan of a, b, c dihedral angles at semiempiric method PM3 level of theory, respectively. For each curve, the stationary points were confirmed by frequency analysis as minima with all-real frequencies and no imaginary frequency, implying no transition state. For the lowest energy conformer, the geometric structure was reoptimized at the HF and DFT levels.




The HF method seemed to be more appropriate than the DFT method for calculation of $^{1}$H-NMR chemical shifts (data for DFT methods not presented in text). Calculations of nuclear shielding of carbons were performed using the GAUSSIAN 09 program with the DFT functional and different basis sets: 6–311G+(d), 6–311G+(d,p) LANL2DZ, DGDZVP, and DGDZVP2. $^{1}$H-NMR chemical shifts were calculated within the GIAO approach by applying the same methods and basis set as used for geometry optimization.

## Results and discussion

Comparing the calculations and the experimental data, the energy values of exo-NH and endo-NH salts were examined to find the most appropriate structure of the molecule. The lowest energy value was observed for the endo-NH salt (exo-NH, −668.005; endo NH, −668.152 hartrees). The experimental data are shown and the occurrence of NH salt because pyrimidines N-$CH_2$ protons have same chemical shift in $^{1}$H-NMR. We studied relationship between the calculation and the experimental data and obtained the following linear formulae: $y = 0.831x + 0.336$ ($r^2 = 0.603$) for 6–311G+(d), $y = 0.812x + 0.228$ ($r^2 = 0.596$) for 6–311G+(d,p), $y = 0.928x - 1.346$ ($r^2 = 0.601$) for LANL2DZ, $y = 0.812x - 0.203$ ($r^2 = 0.553$) for DGDZVP, and $y = 0.868x - 0.024$ ($r^2 = 0.599$) for DGDZVP2. According to these results, the HF/6311G+(d) method has shown better fit to experimental for $^{1}$H chemical shifts.

Theoretical and experimental $^{1}$H-NMR values for (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl)amine hydroiodide are presented in Table 1 and Fig. 1. The calculated results show that the $N_{12}$ atom has the largest negative charge along with its suitable spatial configuration in HF/6–311G+(d), making it a potential site for reaction with metallic cores. Possibility of protonation of the $N_{12}$ atom is the highest. Seeing in Fig. 1 has shown that 6–311G+(d) more suitable for real data for $^{1}$H-NMR.

**Table 1** Theoretical and experimental $^{1}$H-NMR values

| | 6–311(d) | 6.311(d,p) | LANL2DZ | DGDZVP | DGDZVP2 | Experimental |
|---|---|---|---|---|---|---|
| 18 | 7.27 | 6.97 | 6.27 | 6.70 | 7.29 | 6.91 |
| 19 | 7.69 | 7.49 | 7.19 | 7.18 | 7.74 | 6.91 |
| 20 | 7.87 | 7.63 | 6.97 | 7.29 | 7.82 | 7.33 |
| 21 | 8.02 | 7.82 | 7.19 | 7.53 | 8.18 | 7.33 |
| 22 | 2.74 | 2.58 | 1.30 | 2.05 | 2.52 | 4.34 |
| 23 | 2.41 | 2.30 | 1.17 | 1.85 | 2.24 | 4.34 |
| 24 | | | | | | 8.00 |
| 25 | 3.85 | 3.57 | 2.80 | 2.97 | 3.49 | 7.49 |
| 26 | 3.85 | 3.57 | 2.58 | 3.20 | 3.49 | 3.82 |
| 27 | 4.25 | 4.01 | 3.08 | 3.57 | 3.81 | 3.82 |
| 28 | 3.85 | 3.57 | 2.58 | 3.20 | 3.49 | 3.82 |
| 29 | 3.35 | 3.16 | 1.73 | 2.33 | 3.22 | 6.75 |
| 30 | 3.35 | 3.16 | 1.84 | 2.83 | 3.04 | 3.36 |
| 31 | 3.27 | 3.16 | 1.84 | 2.83 | 3.04 | 3.36 |
| 32 | 1.97 | 1.88 | 0.55 | 1.36 | 1.89 | 1.95 |
| 33 | 1.97 | 1.88 | 0.55 | 1.36 | 1.89 | 1.95 |
| 34 | 3.27 | 3.07 | 1.95 | 2.69 | 3.04 | 3.36 |
| 35 | 3.35 | 3.16 | 1.95 | 2.74 | 3.04 | 3.36 |

Figure 2 shows the atom numbering scheme adopted in this study for (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl)amine hydroiodide.

The Mulliken charges of (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl)amine hydroiodide are shown in Fig. 3, while Fig. 4 shows the total electron contours of the compound.

Figure 5 shows the highest occupied molecular orbital (HOMO)−1, HOMO, lowest unoccupied molecular orbital (LUMO), and LUMO+1 orbitals of **2**, calculated at the HF/6–311G+(d) level. The occupied orbitals (HOMO−1 and HOMO) of **2** have a large contribution from the NH group, $OCH_3$ group, and aromatic ring. In addition, the HOMO and LUMO values of the compound are −0.18476 and −0.28414 hartrees, respectively.

In our study, the (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl)amine hydroiodide compound was synthesized and evaluated for its antihistaminic effect on guinea pig trachea. The isolated guinea-pig trachea pre-contracted with histamine was used to study the relaxation of the test material. The isolated constituent, (4-methoxybenzyl)(1,4,5,6-tetrahydropyrimidin-2-yl)amine (2) caused 80 % relaxation of maximal contraction produced by histamine at a concentration of 1,25 mg/ml (cumulative). Relaxant effect of compound (2) on contractions of guinea pig tracheal chains induced by histamine with an $EC_{50}$ of $3.01 \times x10^{-5}$ M (Fig. 6).

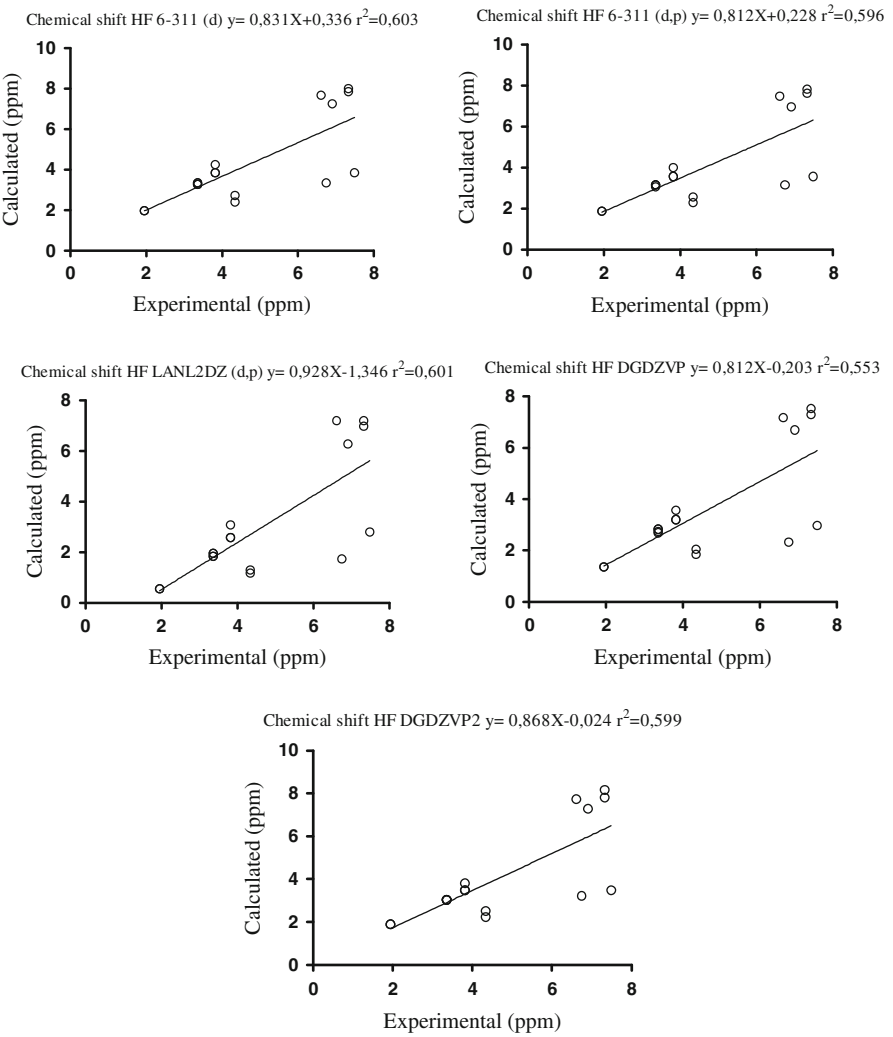



**Fig. 1** $^{1}$H-NMR data theoretical and experimental values of compound 2

Histamine is an important mediator of airway smooth muscle contraction, and bronchial obstruction occurs via H1-receptors. H1-receptor blockade results in bronchodilation [36–38]. The presented data indicate a relaxant effect of (4-methoxybenzyl)(1,4,5, 6-tetrahydropyrimidin-2-yl)amine hydroiodide on histamine-contracted isolated guinea pig tracheal smooth muscle, possibly via histamine H1-receptor antagonistic activity. These results are comparable to those of promethazine, which is known as an H1-receptor antagonist [39]. The present findings reveal that compound (2), via its H1 antihistaminic nature, has potential activity to increase the threshold for bronchoconstriction, leading to bronchodilation.

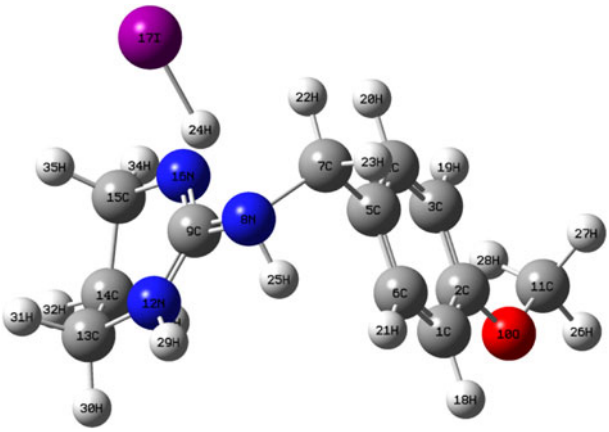
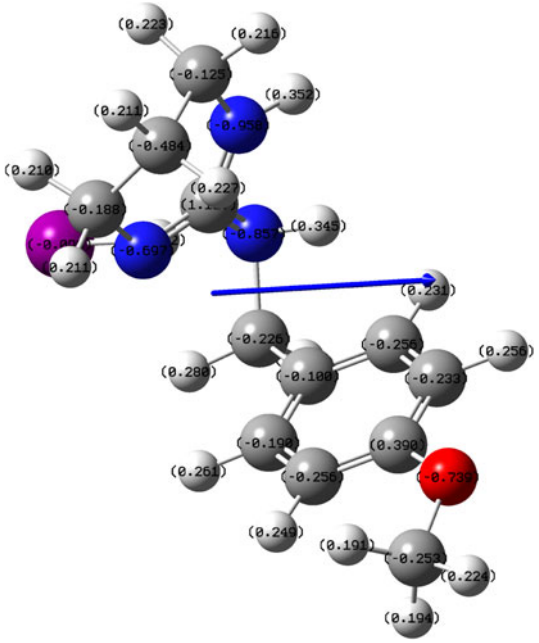



**Fig. 2** Atom numbering scheme for compound 2

**Fig. 3** Mulliken charges of compound 2




Theoretical studies showed that the NH group, $OCH_3$ group, and aromatic ring play critical roles in the antihistaminic activity. The endo-nitrogen atom, however, significantly contributed to the HOMO of the compound, suggesting that it is an additional nucleophilic site for activity.

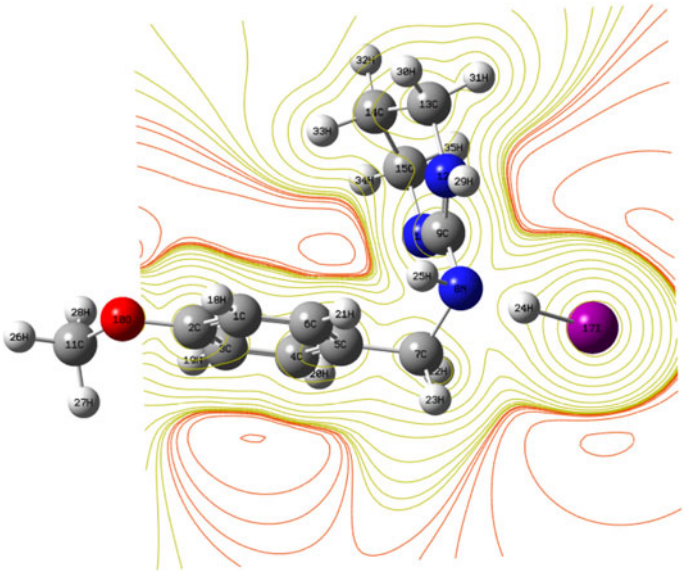


**Fig. 4** Total electron contours map of compound 2

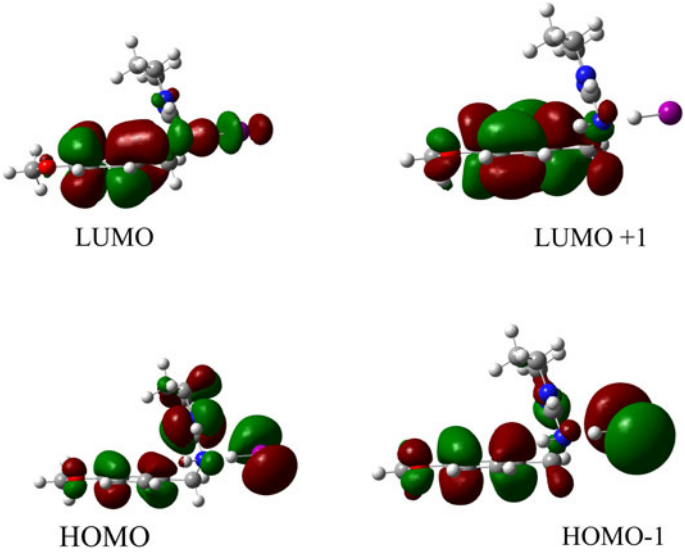



**Fig. 5** Calculated orbital population from HOMO−1 to LUMO+1 of compound 2

These calculations also indicate that the nucleophilic property of 2-amino-1,4,5,6-tetrahydropyrimidines is a critical feature for the antihistaminic activity of these compounds.

It can be suggested that the relaxant effect of the title compound on histamine-induced tracheal contraction may be via its antihistaminic effect.

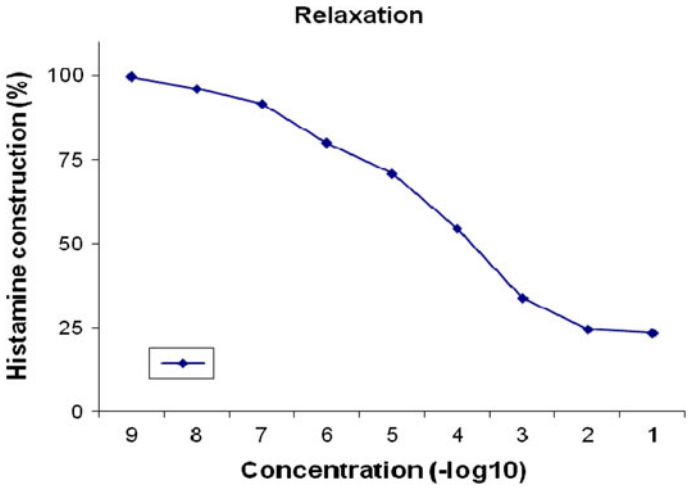



**Fig. 6** The relaxant effect of test materials on contractions of guinea pig tracheal chain induced by histamine (1.25 mM cumulative). $EC_{50} = 3.01 \text{ X } 10^{-5}$ M

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