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**Research Article**

# *N*-((4-DIMETHYLAMINO)PHENYL(HYDROXY)METHYL)MORPHOLINE -4-CARBOTHIOHYDRAZIDE: SYNTHESIS, STRUCTURAL ANALYSIS AND ANTITUMOUR ESSAY

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**ABSTRACT**

*N(4)- substituted thiosemicarbazone was a potential class of organic compounds due to its effective bioactivities. In this study, the condensation of 4-dimethylaminobenzaldehyde (4–DB) and N(4)- morpholinylthiosemicarbazide (MT) was conducted in ethanol/glacial acetic acid as a catalyst with the molar 4–DB - MT ratio of 0.89:1 at 75$^0$C for 90 mins to obtain N-((dimethylamino)phenyl(hydroxy)methyl) morpholine-4-carbothiohydrazide ($H_2K$), instead of the target thiosemicarbazone. The structure of $H_2K$ was analyzed by IR, UV-Vis, $^1$H ,$^{13}$C-NMR, HSQC, HMBC, and HRMS. $H_2K$ existed the thioketone form in the solid state. In ethanol, there was an equilibrium of thioketone and thiol of $H_2K$. The literature mechanism of the condensation showed that $H_2K$ was supposed to be an intermediate prior to the dehydration leading to imine formation. The antitumour performance of $H_2K$ for lung cancer ($IC_{50}$ = 9.37 µg/mL) was greater than that of liver cancer ($IC_{50}$ = 40.95 µg/mL). Therefore, $H_2K$ possesses a comparable antitumour performance in comparison with thiosemicarbozones.*

***Keywords:*** antitumour; morpholine; condensation; thioketone; thiol

## 1. Introduction

Thiosemicarbazone (TSC) prepared since the 20$^{th}$ century (Bavin et al., 1951; Wallace et al., 1956; French, & Blanz, 1965) has attracted many scientists because of outstanding bioactivities such as antifungal, antivirus, antibacterial, and antitumour. TSC possesses the antitumour selectivity because TSC molecules can prevent the translation and transcription of distorted DNA through the coordination of the donor atoms (nitrogen and sulfur atoms) and basic nucleotides (Sreekanth, 2003; Rapheal, 2006; Fatondji et al., 2013; El-Sawaf et al., 2018).

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In 2017, Duong Ba Vu and his team synthesized 4-dimethylaminobenzaldehyde-*N*(4)-morpholinylthiosemicarbazone (4–DMT) by the condensation of 4-dimethylaminobenzaldehyde (4–DB) and *N*(4)-morpholinylthiosemicarbazide (MT). However, the obtained 4 –DMT was considered as a mixture of intermediates, thioketone, and thiol of 4-DMT (Duong, Trang, & Tran, 2017). In this study, the synthetic process of 4-DMT (Duong et al., 2017) and isolated *N*-((dimethylamino)(hydroxy)methyl)morpholine-4-carbothiohydrazide ($H_2K$) as an intermediate was redesigned (Figure 1). $H_2K$ was characterized its structure and investigated its antitumour performance in comparison with the mixture of 4-DMT.

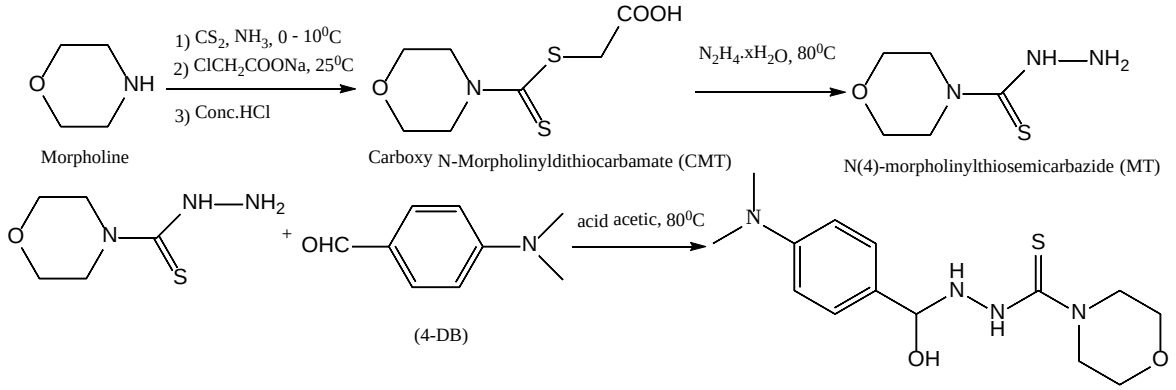


***Figure 1.*** *The scheme of $H_2K$ synthesis*

## 2. Experiment

### *2.1. Chemicals and equipment*

Sodium chloroacetate and hydrazine hydrate were purchased from Aldrich – Sigma, USA. Morpholine and 4-dimethylaminobenzaldehyde were produced from Merck, Germany**.** Carbon disunfide, hydrochloride, ethanol, glacial acetic acid were prepared from Xilong, China.

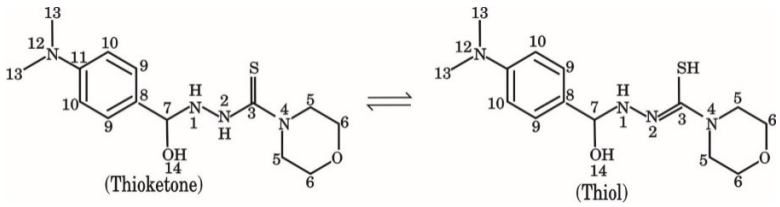


***Figure 2.*** *The equilibrium of thioketone and thiol of $H_2K$ (the target compound in this study) in solution*

Fourier Transform Infrared (FT-IR) analysis (Shimadzu FT-IR-8400S) was operated in the range of 4000-450 $cm^{-1}$ using compressed KBr pellets. Ultraviolet-visible light absorbance measurements were performed by using a Perkin-Elmer Lambda 25 UV-Vis Spectrometer in the range of 200-700 nm in absolute ethanol. A Gallenkamp MPD-350 was used to determine melting point temperatures. Nuclear magnetic resonance (NMR)

spectra were recorded by using a Bruker 500 MHz (in d6-dimethylsulfoxide, DMSO-d6), and high-resolution mass spectrometry positive spectra obtained from a Varian 910 MS.

### *2.2. Synthesis of $H_2K$*

The synthetic processes of intermediates were referred from Duong Ba Vu et al., 2017. 1,0 g of *N*(4)-morpholinylthiosemicarbazide (MT) in 20 mL of ethanol and three drops of glacial acetic acid (mixture 1) were refluxed to form a homogenous solution. 0.9633 g of 4-dimethylaminobenzaldehyde (4-DB) in 15 mL of hot ethanol were added wisely into the mixture 1 at 75$^o$C. After 90 mins, the yellow precipitate ($H_2K$) was separated from the solution. $H_2K$ was filtered and recrystallized from ethanol. The yield: 85%. $t^0_{melting}$ = 204$^0$C-207$^0$C; FT IR ($\nu$, cm$^{-1}$): 3531, 3367, 3003, 2903, 1613, 1034, 886; UV – Vis ($\lambda_{max}$, nm, MeOH): 202, 259, 364, 506; $^1$H-NMR (DMSO-d$_6$, 500Hz, $\delta$, ppm): 2.96 (6H, *s*, -CH$_3$); 3.63 (4H, *t*, H-morpholine); 3.85 (4H, *t*, H-morpholine); 6.72 (2H, *d*, H-Ar); 7.59 (2H, *d*, H-Ar); 7.97 (1H, *s*, CH-OH); 9.91(1H, *s*, NH); 10.05 (1H, *s*, NH); 11.59 (1H, *s*, OH); $^{13}$C –NMR (DMSO-d$_6$, $\delta$, ppm): 39.5; 48.5; 65.7; 111.7; 121.0; 128.6; 144.2; 151.5; 176.9; 182.4; HRMS (MeOH, MS(+), m/z): 147.8; 205.8; 366.8; 332.9; 279.8.

## 3. Results and discussion

***Table 1.*** *The key data of 4-DB, MT, $H_2K$ and 4-DMT in FTIR, and UV-Vis*

| Sample | Wavenumber (cm$^{-1}$) (FT IR) | | | | | | | $\lambda_{max}$ (nm) (UV-Vis) | | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| | **O-H** | **N-H** | **C=O** | **C=N** | **N-N** | **C=S** | **S-H** | **π*←π** | **π*←n** | |
| 4-DB | - | - | 1681 | - | - | - | - | - | - | This study |
| MT | - | 3459 | - | - | 1039 | 1358 887 | - | - | - | This study |
| 4-DMT | - | 3163 | - | 1520 | 1018 | 1334 887 | 2363 | 205; 235 | 365 | (Duong Ba Vu et al., 2017) |
| $H_2K$ | 3531 | 3367 | - | - | 1034 | 1341 886 | - | 202; 259 | 364; 506 | This study |

For FTIR spectrum of $H_2K$, there was no absorption at 2700 cm$^{-1}$ and 1690-1680 cm$^{-1}$ which were assigned to stretching vibration of C=O of aldehyde. The broad absorption at 3531 cm$^{-1}$ showed the stretching vibration of -OH. The correlation of $H_{14}$ (-OH) and $C_7$ was also recorded by HMBC of $H_2K$. The result is that MT was condensed successfully with 4-DB to form a product containing hydroxyl group.

The absorption at wavenumber of 1034 cm$^{-1}$ was assigned to the vibration of N-N. The vibration of C=S was observed at 1341 cm$^{-1}$ and 886 cm$^{-1}$ C=S, whereas the vibration

of S-H was not recorded at 2500 $cm^{-1}$. Based on the analysis of $^{13}$C-NMR and HMBC, the correlation of H5 and C3(=S) enabled to assign the resonance peak with the chemical shift of $\delta$ = 180 ppm for thioketone. The peak at $\delta$ = 178 ppm was expected to be the carbon atom of C-SH. Thus, $H_2K$ existed thioketone form in its solid state, while thioketone and thiol can set up an equilibrium in the solution. This transformation did not affect the chemical shifts of protons in $^1$H NMR of $H_2K$. There are obviously 9 resonance peals represented 22 protons of a $H_2K$ molecule. It was because of the lack of a conjugate system -C=N-N=C-(SH)- of a normal thiosemicarbazone. As a result, the predictive skeleton of $H_2K$ was R-CH(OH)-NH-NH-C(=S)-R'. This structure was confirmed by the fragmentation analysis in MS of $H_2K$ (figure 3). The fragments were pseudo-molecular ion peaks stabilized by ion $Na^+$, ion $H^+$, morpholine, or solvent molecules.

In $^1$H-NMR and HMQC, there were two peaks at $\delta$ = 3.63 ppm and 3.85 ppm (4H, *triplet*), representing protons in morpholino moiety. This pattern demonstrated that the axial and equatorial protons were chemically equivalent. Likely the observation from Duong Ba Vu et al. (2017), two conformations of morpholino moiety was in an equilibrium. The chemical shift $\delta$ =3.85 ppm and $\delta$ = 3.65 ppm were assigned to $H_5$ and $H_6$ respectively due to the correlation of $H_5$ and C=S.

The UV-Vis spectra of $H_2K$ in ethanol showed two absorption bands: the π*←n transition bands ($\lambda$ =364 nm (lg $\varepsilon$ = 4.38); $\lambda$ =507 nm (lg $\varepsilon$ = 3.61)) owing to the excitation of electrons from MO-n of O, N, S to MO- π*; the π*←π transition bands ($\lambda$ =202 nm (lg $\varepsilon$ = 4.44); $\lambda$ = 259 nm (lg $\varepsilon$ = 4.1)) due to the π electrons. The UV-Vis of (Duong et al., 2017) did not observe the absorption at 507 nm. It can be interpreted that the red shift occurred because of the stronger hydrogen bonding of OH and ethanol, in comparison with the strength of the hydrogen bonding of NH and ethanol.

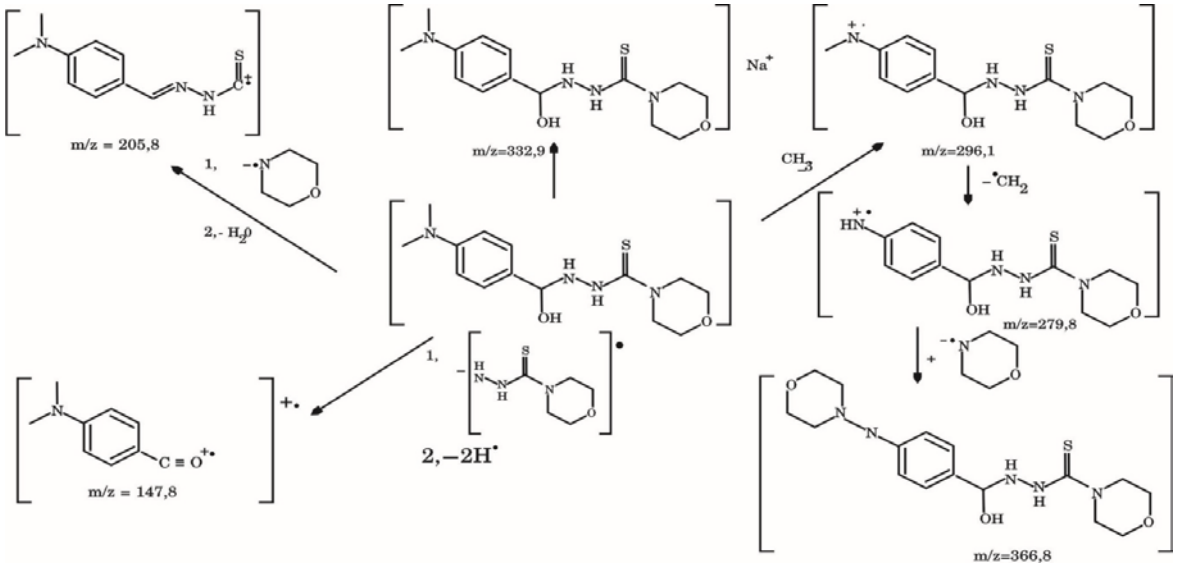


***Figure 3.*** *The fragmentation in MS of $H_2K$*

According to the literature, the condensation mechanism to synthesize 4-DMT was suggested as Figure 4:

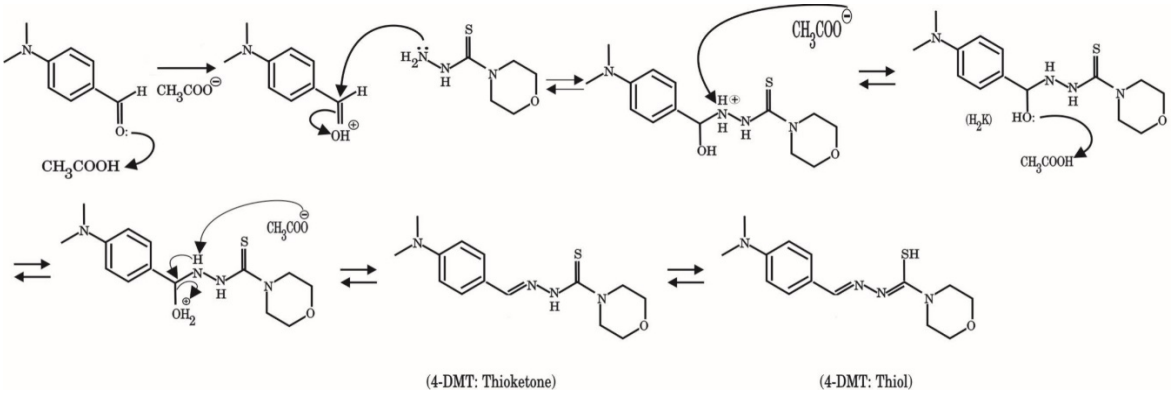


***Figure 4.*** *The mechanism of formation and transformation of $H_2K$*

The observation of mechanism indicates that $H_2K$ is an intermediate for converting to the target thiosemicarbazone. According to Duong Ba Vu et al. (2017), the obtained mixture can compose of thioketone-TSC, thiol-TSC, and $H_2K$ (with the low abundance). Therefore, the experimental analysis and the literature revision enabled to conclude the structure of $H_2K$ as predicted in Figure 2.

The bioactive results from Hep-G2 and A549 showed that $H_2K$ possessed the greater antitumour for lung cancer cells ($IC_{50}$ = 9.37 µg/ml) than that of liver cancer cells ($IC_{50}$ = 40.95 µg/ml). Furthermore, $H_2K$ can prevent the growth of lung cancer cells twice more than 4-DMT. Thus, $H_2K$ can be studied as a bioactive ligand for antitumour complexes.

***Table 2.*** *The antitumor results of $H_2K$ and 4 – DMT*

| **Sample** | **Initial concentration (µg/ml)** | **$IC_{50}$; µg/mL** | | **Ref.** |
|---|---|---|---|---|
| | | **Hep-G2** | **A549** | |
| $H_2K$ | 100 | 40.95 | 9.37 | This study |
| 4 –DMT | 100 | > 100 | - | Duong Ba Vu et al. (2017) |

## 4. Conclusion

$H_2K$ was considered as an intermediate for the condensation of 4-dimethylaminobenzaldehyde (4–DB) and *N*(4)- morpholinylthiosemicarbazide (MT). $H_2K$ existed as thioketone in the solid state. The equilibrium of thioketone and thiol can be observed in the solution. The basic sites such as oxygen atoms of OH, nitrogen atoms of NH-NH and sulfur atoms of C=S, $H_2K$ play a role as potential ligand for synthesis of antitumour complexes in next studies, especially anti-Hep-G2 (lung cancer cells).

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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# *N*-((4-DIMETHYLAMINO)PHENYL(HYDROXY)METHYL)MORPHOLINE -4-CARBOTHIOHYDRAZIDE: TỔNG HỢP, NGHIÊN CỨU CẤU TRÚC VÀ THĂM DÒ HOẠT TÍNH ỨC CHẾ TẾ BÀO UNG THƯ

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## TÓM TẮT

*Thiosemicarbazone với nhóm thế N(4) là một lớp chất hữu cơ tiềm năng trong nghiên cứu các loại thuốc có hoạt tính sinh học cao. Trong nghiên cứu này, phản ứng ngưng tụ giữa 4-dimethylaminobenzaldehyde (4–DB) và N(4)- morpholinylthiosemicarbazide (MT) được tiến hành trong dung môi ethanol với xúc tác glacial acetic acid, tỉ lệ mol của 4–DB - MT là 0,89:1 ở 75$^0$C trong 90 phút. Sản phẩm thu được là N- ((dimethylamino)phenyl(hydroxy)methyl) morpholine-4-carbothiohydrazide ($H_2K$), thay vì thiosemicarbazone theo kết quả thông thường. Cấu trúc phân tử của $H_2K$ được phân tích và quy kết bằng IR, UV-Vis, $^1$H ,$^{13}$C-NMR, HSQC, HMBC and HRMS. $H_2K$ tồn tại dạng thioketone trong pha rắn. Trong dung môi ethanol, thioketone chuyển hóa một phần thành thiol. Dựa vào cơ chế lí thuyết của phản ứng ngưng tụ tạo imine, $H_2K$ được xem như hợp chất trung gian trước khi tham gia quá trình tách một phân tử nước để tạo thành thiosemicarbazone. $H_2K$ có khả năng ức chế sự phát triển tế bao ung thư phổi ($IC_{50}$ = 9,37 µg/mL) hiệu quả hơn so với tế bào ung thư gan ($IC_{50}$ = 40,95 µg/mL).*

***Từ khóa:*** ức chế tế bào ung thư; morpholine; phản ứng ngưng tụ; thioketone; thiol