VILSMEIER-HAACK SYNTHESIS OF NEW STEROIDAL PYRAZOLES

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Keywords: Thiosemicarbazone, pyrazole, Vilsmeier-Haack, acetamide, $POCl_3$.

A novel expeditious and convenient synthesis of hitherto unknown 5α-cholestano [6,7-c]-5'-methyl-1'-carbothioic acid amide pyrazoles based on the reaction of 5α-cholestan-6-one thiosemicarbazones with modified Vilsmeier-Haack reagent (H_3C-CO-NH_2/$POCl_3$) is described. The compounds presented here are novel scaffolds and have not been described before. Structural assignment of these newly synthesized compounds was performed by IR, ^{1}HNMR, ^{13}CNMR, MS and analytical data. A general mechanistic scheme for these reactions is also suggested based on the current and previous data.

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Introduction

Pyrazole moiety, being called as pharmacophore, plays an important role in many biologically active compounds and therefore represents an interesting template for combinatorial as well as medicinal chemistry.[1-5] In addition pyrazoles have played a vital role in developing the theory in heterocyclic chemistry and are also used extensively as useful synthons in organic synthesis.[6] These derivatives have wide spread biological activities such as anticancer, analgesic, anti-inflammatory, antimicrobial, antiviral, anticonvulsant, antihistaminic, and anti-HIV.[6-17] The recent success of pyrazole COX-2 inhibitor has further highlighted the importance of these heterocycles in medicinal chemistry.[6] Some of the biologically active steroids fused with heterocycles are shown in Figure 1.a vital role in developing the theory in heterocyclic chemistry and are also used extensively as useful synthons in organic synthesis.[6] These derivatives have wide spread biological activities such as anticancer, analgesic, anti-inflammatory, antimicrobial, highlighted the importance of these heterocycles as antiviral, anticonvulsant, antihistaminic, and anti-HIV.[6-17] The recent success of pyrazole COX-2 inhibitor has further highlighted the importance of these heterocycles in medicinal chemistry.[6] Some of the biologically active steroids fused with heterocycles are shown in Figure 1.

Since the discovery of the Vilsmeier-Haack reagent (halomethyleniminium salt) in 1927, formed from the interaction of a dialkyl formamide (e.g. DMF) with phosphorus oxychloride ($POCl_3$) has attracted the attention of synthetic organic chemists.[18] It is one of the most commonly used reagents for the introduction of an aldehydic (CHO) group into electron rich aromatic systems.[19] However, the scope of the Vilsmeier reagent is not confined to the aromatic formylation reaction alone. A wide variety of alkene derivatives,[20] carbonyl compounds,[21] activated methyl and methylene groups[22] exhibit reactivity towards the Vilsmeier reagent. In addition to the carbon nucleophiles, some oxygen and nitrogen nucleophiles[23,24] are also reactive towards Vilsmeier reagent. Numerous transformations of the iminium salts into products other than aldehydes have been achieved[25,26] and these transformations enhance the scope and versatility of the Vilsmeier–Haack reaction. In continuation of our previous work[27] and following interest on the Vilsmeier-Haack reaction, we hereby report an effort to introduce the methyl group instead of an aldehydic group into the steroidal system by modifying the iminium cation, formed by the expeditious reaction of acetamide and $POCl_3$.

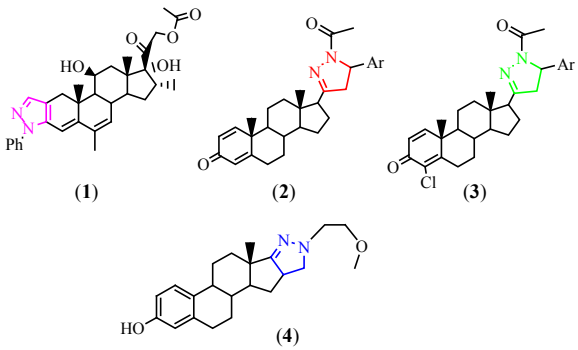


Figure 1. Some biologically active steroidal appended pyrazoles

Experimental

Materials and instruments

All the reagents and solvents were obtained from best known commercial sources and were freshly distilled. Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Pye Unicam SP3-100 Spectrophotometer and values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ on a JEOL Eclipse (400 MHz) instrument with TMS as internal standard and values are given in ppm (δ). Mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer. Thin layer chromatography (TLC) plates

were coated with silica gel G and exposed to iodine vapours to check the progress of reaction. Anhydrous sodium sulphate was used as a drying agent.

General procedure for the synthesis of steroidal pyrazoles (4-6)

To the solution of steroidal thiosemicarbazones[21] (**1-3**) (1 mmol) in CH_3CN (10 mL), was added acetamide (1 mmol) under ice-cold condition. $POCl_3$ (1 mmol) was then added with stirring at such a rate that the temperature of the reaction mixture did not exceed 10 °C. After complete addition, the reaction-mixture was allowed to attain room temperature and stirred for about 1-3 h. After ensuring the completion of reaction (TLC), the contents were poured into crushed ice and into and left overnight in a refrigerator. The precipitate thus obtained was filtered, washed with water, dried and purified by recrystallization from methanol to afford 5α-cholestano[6,7-c]-5'-methyl-1'-carbothioic acid amide pyrazole derivatives (**4-6**).

3β-Acetoxy-5α-cholestano[6,7-c]-5'-methyl-1'-carbothioic acid amide pyrazole (4)

Yield 65 %; mp: 147-149 °C; Anal. Calcd. for $C_{32}H_{51}N_3O_2S$: C, 70.89, H, 9.19, N, 7.55; found; C, 70.97, H, 9.42, N, 7.76; IR (KBr) ν cm^{-1}: 3390 (NH_2), 1714 ($OCOCH_3$), 1655 (C=N), 1632 (C=C), 1376 (C-N), 1269 (C=S), 1210 (C-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.2 (s, 2H, NH_2, exchangeable with D_2O), 4.7 (m, 1H, $C_3\alpha$-H, W ½ = 15 Hz), 2.3 (s, 3H, CH_3), 2.03 (s, 3H, $OCOCH_3$), 1.18 (s, 3H, C_{10}-CH_3), 0.70 (s, 3H, C_{13}-CH_3), 0.97 & 0.83 (other methyl protons); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 181.2 (*C*=S), 171.0 (OAc), 148.3 (C_6), 134 (C_5'), 119.3 (C_7), 75.1 (C_3), 40.5 (C_5), 39.52, 37.98, 36.70, 35.76, 30.73, 30.08, 28.11, 28.07, 24.04, 23.86, 22.89, 22.63, 21.57, 19.09, 18.70, 13.21, 12.08; ESI MS: m/z 541[$M^{+\cdot}$].

3β-Chloro-5α-cholestano[6,7-c]5'-methyl-1'-carbothioic acid amide pyrazole (5)

Yield 65 %; mp: 156-158 °C; Anal. Calcd. for $C_{30}H_{48}N_3ClS$: C, 69.49, H, 9.11, N, 8.02; found; C, 69.63, H, 9.28, N, 8.12; IR (KBr) ν cm^{-1}: 3410 (NH_2), 1650 (C=N), 1625 (C=C), 1378 (C-N), 1275 (C=S), 756 (C-Cl); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.8 (s, 2H, NH_2, exchangeable with D_2O), 3.9 (m, 1H, $C_3\alpha$-*H*, W ½ = 17 Hz), 2.4 (s, 3H, CH_3), 1.19 (s, 3H, C_{10}-CH_3), 0.75 (s, 3H, C_{13}-CH_3), 0.97 & 0.80 (other methyl protons); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 184.2 (*C*=S), 144.5 (C_6), 132 (C_5'), 119.2 (C_7), 59.6 (C_3), 42.2 (C_5), 42.92, 39.32, 38.13, 36.64, 35.72, 30.68, 30.14, 30.08, 28.42, 28.12, 24.18, 23.56, 22.62, 22.43, 21.47, 18.68, 13.23; ESI MS: m/z 515/517 [$M^{+\cdot}$].

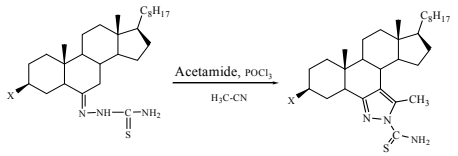
5α-Cholestano[6,7-c]5'-methyl-1'-carbothioic acid amide pyrazole (6)

Yield 60 %; mp: 119-121 °C; Anal. Calcd for $C_{30}H_{49}N_3S$: C, 74.40, H, 10.07, N, 8.60; found; C, 74.53, H, 10.14, N, 8.69; IR (KBr) ν cm^{-1}: 3393 (NH_2,), 1652 (C=N) 1620 (C=C), 1375 (C-N), 1275 (*C*=S); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.6 (s, 2H, NH_2, exchangeable with D_2O), 2.4 (s, 3H, CH_3), 1.19 (s, 3H, C_{10}-CH_3), 0.75 (s, 3H, C_{13}-CH_3), 0.96 and 0.83 (other methyl protons); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 179.2 (C=S), 150.1 (C_6), 137 (C_5'), 118 (C_7), 41.8 (C_5), 42.76, 39.45, 38.24, 36.53, 35.87, 30.52, 30.34, 30.12, 28.57, 28.07, 24.38, 23.82, 22.46, 22.36, 21.82, 18.48, 17.62, 13.27, 12.16; MS: m/z 483[$M^{+\cdot}$].

Results and Discussion

Chemistry

Development of highly functional molecules from simple building blocks has always been the aim of synthetic chemists. With this aim, we here report a new route for the synthesis of novel steroidal pyrazoles (**4-6**) from steroidal thiosemicarbazones (**1-3**) by using the Vilsmeier-Haack protocol, during which the steroidal thiosemicarbazones (**1-3**) reacts with iminium cation (formed by the reaction of acetamide and $POCl_3$ under cold conditions), afforded steroidal pyrazoles (**4-6**) (Scheme 1). The yield of steroidal pyrazoles (**4-6**) was in the range of 60-65 %. A probable mechanism for the formation of compounds (**4-6**) is shown in Scheme 2.

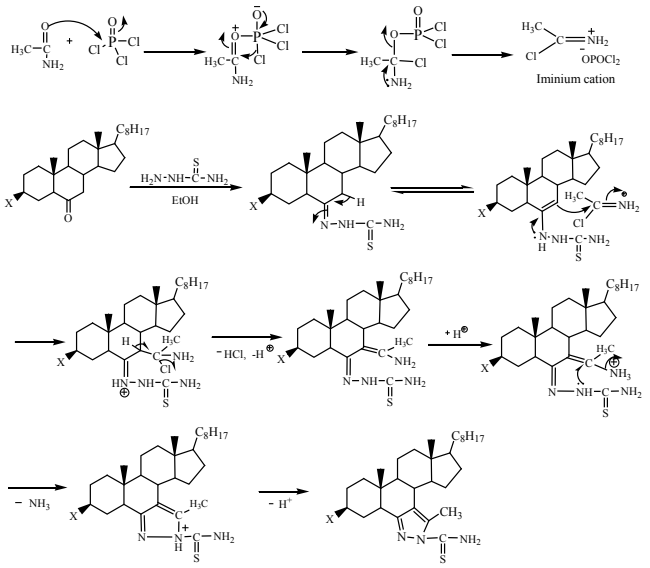


X
OAc (**1**)
Cl (**2**)
H (**3**)

X
OAc (**4**)
Cl (**5**)
H (**6**)

Scheme 1. Showing the formation of steroidal pyrazoles **4-6**

The selected diagnostic bands of IR spectra of synthesized products provide useful information for determining structures of the steroidal pyrazole derivatives. All the compounds (**4-6**) show a band in the range of 3390-3410 cm^{-1} assigned to (NH) while the bands in the range of 1655-1650, 1632-1620, 1378-1375, 1269-1275 cm^{-1} were be ascribed to (C=N), (C=C), (C-N) and (C=S) respectively. The formation of steroidal pyrazoles (**4-6**) was further confirmed with the NMR studies where the assignments of signals are based on the chemical shift and intensity pattern. In ^{1}HNMR spectra the compounds (**4-6**) exhibited singlet for three protons at δ 2.3-2.4 (CH_3) and two proton singlet (exchangeable with D_2O) at δ 8.2-7.6 (NH_2). Other peaks were observed at δ 1.18, 0.70, 0.97 and 0.83 indicating the presence of angular and side chain methyl groups. ^{13}CNMR signals are in good agreement with proposed structures of synthesized compounds. All the compounds exhibited signals at δ 184-179, 150-144, 137-132,119-118 due to *C*=S, *C*=N, *C*-N and C=C respectively. The distinctive signals were observed in the mass spectra of compounds (**4-6**) which followed the similar fragmentation pattern. The molecular ion peaks ($M^{+\cdot}$) for compounds (**4-6**) were observed at m/z 541, 515/517 and 483 respectively.



Scheme 2. Showing the reaction mechanism for the formation of steroidal pyrazoles

The mechanism for the formation of steroidal pyrazoles (4-6) involves the formation of an iminium cation by the reaction of acetamide and phosphorus oxychloride. The steroidal thiosemicarbazones undergo keto-enol tautomerization before reacting with iminium cation. Facilitated by the migration of the lone pair of electron from nitrogen, the attack of the double bond to the iminium cation occur which in turn expels hydrogen chloride and ammonia, leading to the formation of steroidal pyrazoles

Conclusion

In conclusion, we have developed an efficient synthetic approach for the synthesis of a novel class of steroidal pyrazoles via modified Vilsmeier reagent (acetamide/$POCl_3$). This is a simple, mild and straightforward reaction, which is completed in a short span of time. The novelty of the entire process lies in the Vilsmeier cyclization of cholestane-6-one thiosemicarbazones into steroidal pyrazoles, which, to the best of our knowledge, is unprecedented. Further studies to broaden the scope towards the synthesis of the novel steroidal derivatives are under investigation in our laboratory.

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