J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 49 (1): 103-108, 2025
Doi: 10.33483/jfpau.1571272

ORIGINAL ARTICLE / ÖZGÜN MAKALE

REGIOISOMERIC *N*-ALKYLATION OF SOME INDAZOLES

BAZI İNDAZOLLERİN REGİOİZOMERİK N-ALKİLASYONU

Fatima DOGANC[1], Hakan GOKER[1]*

[1]Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06560, Ankara, Türkiye

ABSTRACT

Objective: *Indazole scaffold have two interconvertible tautomeric forms. Based on our previous studies, regioisomeric N-alkylation of some indazole analogs was synthesized in this study and their structures were elucidated by 2D NMR methods.*
Material and Method: *Regioisomers were resolved for N-benzylations and alkylation of some non-substituted and substituted indazoles, under basic conditions (K_2CO_3) in DMF.*
Result and Discussion: *It was observed that, their occurrence ratios of N1 : N2 is almost equal (50%). Their structures were established by combination of ^{1}H-^{1}H NOE (Nuclear Overhauser Effect Spectroscopy, NOESY) and HMBC (Heteronuclear Multiple Bond Correlation) NMR methods.*
Keywords: *HMBC, indazole (1,2-benzodiazole), NOESY, regioisomers*

ÖZ

Amaç: *İndazol halkası iki tautomerik forma sahiptir. Önceki çalışmalarımızda yola çıkarak, bu çalışmada bazı indazol analoglarının regioizomerik N-alkilasyonu sentezlendi ve yapıları 2D NMR yöntemleriyle aydınlatıldı.*
Gereç ve Yöntem: *Bazı non-sübstitüe ve sübstitüe indazollerin, DMF içinde bazik koşullar (K_2CO_3) altında N-alkilasyonu yoluyla regioizomerik N-alkilasyon türevleri elde edildi.*
Sonuç ve Tartışma: *N1 : N2 oluşum oranlarının neredeyse eşit olduğu (%50) gözlemlendi. Moleküllerin yapıları, ^{1}H-^{1}H NOE (Nuclear Overhauser Effect Spectroscopy, NOESY) ve HMBC (Heteronuclear Multiple Bond Correlation) NMR yöntemleri ile aydınlatıldı.*
Anahtar Kelimeler: *HMBC, indazol (1,2-benzodiazol), NOESY, regioizomer*

INTRODUCTION

Indazoles are a significant class of heterocyclic compounds with a diverse range of biological and pharmaceutical applications. Indazole derivatives play a crucial role in pharmacology as they form the core structure of various drug molecules, including Granisetron, a 5HT3 receptor antagonist used as an antiemetic in cancer chemotherapy, Benzydamine, an anti-inflammatory agent, and the anti-cancer drug Pazopanib [1,2]. Indazoles are bearing a bicyclic ring structure made up of a pyrazole and a benzene ring. Indazole may exist in two tautomeric forms resulting from the transfer of a proton between the two nitrogen atoms, a process described as prototropic annular tautomerism (Figure 1). Studies on molecular refractivity indicate that non-substituted indazole predominantly exists as the 1*H*-tautomer (I). Research has shown that the 1*H* form is generally more stable than the 2*H* form in both gas-phase solutions and solid-state derivatives [3,4]. However, Alkorta and Elguero demonstrated through theoretical

* **Corresponding Author / Sorumlu Yazar:** Hakan Göker
e-mail / e-posta: goker@ankara.edu.tr, **Phone / Tel.:** +903122033013

Submitted / Gönderilme : 21.10.2024
Accepted / Kabul : 10.11.2024
Published / Yayınlanma : 20.01.2025

calculations that in certain instances, the 2*H*-tautomer is more stable than the 1*H*-tautomer [5].

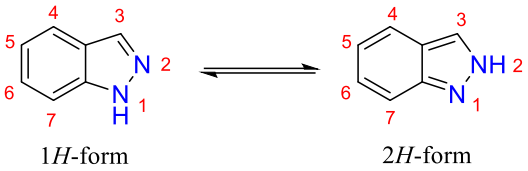


Figure 1. Annular prototropic tautomerism of indazole

The relocation is completely lost when the hydrogen on the pyrazole is replaced with any alkyl groups in indazoles.

In our recently published papers, we have characterized the occurrence and structures of some regioisomers of imidazopyrimidines, imidazopyridines, imidazopyrazines, benzimidazoles and indazoles [6-10]. For this purpose, we used advance 2D-NMR techniques for the structural elucidation. In continuation of these works, we now report, substituted indazole with some alkyl halids, for investigation of the possible regioisomers.

MATERIAL AND METHOD

Uncorrected melting points were measured on an Büchi B-540 capillary melting point apparatus. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded employing BRUKER AVANCE NEO 500 MHz FT spectrometer, chemical shifts (δ) are in ppm relative to TMS. The samples (5-10 mg) were prepared in 0.75 ml of $CDCl_3$.The liquid chromatography mass spectrometry (LC-MS) spectra were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC (Waters Corporation), using the ESI (+) method with a C-18 column (XTerra®, 4.6 X 250 mm, 5 µm).

6-Fluoro-1-(4-methoxybenzyl)-1*H*-indazole (Ia) and 6-Fluoro-2-(4-methoxybenzyl)-2*H*-indazole (Ib)

K_2CO_3 (0.152 g, 1.1 mmol) was added to a suspension of the 6-fluoro-1*H*-indazole (0.136 g, 1 mmol) in DMF (1 ml) and stirred. One hour later, 4-methoxybenzyl chloride (0.172 g, 1.1 mmol) was added. After overnight stirring at room temperature, water was added and oily precipitate was separated. Crude product was purified by column chromatography.

Purification (EtOAc : *n*-Hexane 10 : 100) first afforded the compound **Ia**, yield 0.066 g, 25.8%, mp : 52-54°C. **^{1}H-NMR** δ ppm ($CDCl_3$) : 3.79 (s, 3H, -OC*H*$_3$), 5.49 (s, 2H, *N*-C*H*$_2$), 6.85-6.87 (d, 2H, *J* = 8.6 Hz, H-3',5'), 6.9-6.94 (m, 1H, H-5), 7.00 (broad d,1H, *J* = 9.2 Hz, H-7), 7.18-7.20 (d, 2H, *J* = 8.6 Hz, H-2',6'), 7.66-7.69 (m, 1H, H-4), 8.02 (s, 1H, H-3) ; **COSY** : [H-2',6' / H-3',5'], [H-5 / H-4] ; **NOESY** : [N-C*H*$_2$ / H-7], [N-C*H*$_2$ / H-2',6'], [OC*H*$_3$ / H-3',5'] ; **^{13}C-NMR**, **HSQC** & **HMBC** δ ppm ($CDCl_3$) : 162.1 (d, *J* = 242 Hz, C-6), 159.32 (C-4'), 139.69 (d, *J* = 12.5 Hz, C-7a), 133.48 (C-3), 128.67 (C-2',6'), 128.47 (C-1'), 122.5 (d, *J* = 11 Hz, C-4), 121.27 (C-3a), 114.2 (C-3',5'), 110.6 (d, *J* = 25.9 Hz, C-5), 95.1 (d, *J* = 26 Hz, C-7), 55.26 (-OC*H*$_3$), 52.73 (*N*-*C*H$_2$) ; **MS** (ESI+) m/z : 257 (M+H, 44%), 121 (M+H, 100%), $C_{15}H_{13}FN_2O$.

Continued elution with (EtOAc : *n*-Hexane 12 : 100) provided **Ib**, yield 0.062 g, 24.2%, mp : 84-87°C. **^{1}H-NMR** δ ppm ($CDCl_3$) : 3.81 (s, 3H, -OC*H*$_3$), 5.51 (s, 2H, *N*-C*H*$_2$), 6.87-6.89 (m,1H,H-5), 6.91 (d, 2H, *J* = 8.6 Hz, H-3',5'), 7.27 (d, 2H, *J* = 8.6 Hz, H-2',6'), 7.32 (dd, 1H, *J* = 10.1 & 2.1 Hz, H-7), 7.58 (dd, 1H, *J* = 9.1 & 5.4 Hz, H-4), 7.85 (s, 1H, H-3) ; **COSY** : [H-2',6' / H-3',5'], [H-5 / H-4] ; **NOESY** : [N-C*H*$_2$ / H-3], [N-C*H*$_2$ / H-2',6'], [OCH$_3$ / H-3',5'] ; **^{13}C-NMR**, **HSQC** & **HMBC** δ ppm ($CDCl_3$) : 161.6 (d, *J* = 241 Hz, C-6), 159.8 (C-4'), 148.6 (d, *J* = 13.7 Hz, C-7a), 129.7 (C-2',6'), 127.4 (C-1'), 123 (C-3), 121.85 (d, *J* = 10.4 Hz, C-4), 119.19 (C-3a), 114.6 (C-3',5'), 113.2 (d, *J* =28 Hz, C-5), 100.7 (d, *J* = 23.6 Hz, C-7), 57.09 (*N*-*C*H$_2$), 55.32 (OC*H*$_3$) ; **MS** (ESI+) m/z : 257 (M+H, 55%), 121 (M+H, 100%), $C_{15}H_{13}FN_2O$.

Methyl 1-(4-*tert*-butylbenzyl)-1*H*-indazole-5-carboxylate (**IIa**) **and Methyl 2-(4-*tert*-butylbenzyl)-2*H*-indazole-5-carboxylate** (**IIb**)

K_2CO_3 (0.152 g, 1.1 mmol) was added to a suspension of methyl 1*H*-indazole-5-carboxylate (0.176g, 1 mmol) in DMF (1 ml) and stirred. One hour later, 4-*tert*-butylbenzyl bromide (0.25 g, 1.1 mmol) was added. After overnight stirring at room temperature, water was added and precipitate was filtered. Crude product was purified by column chromatography.

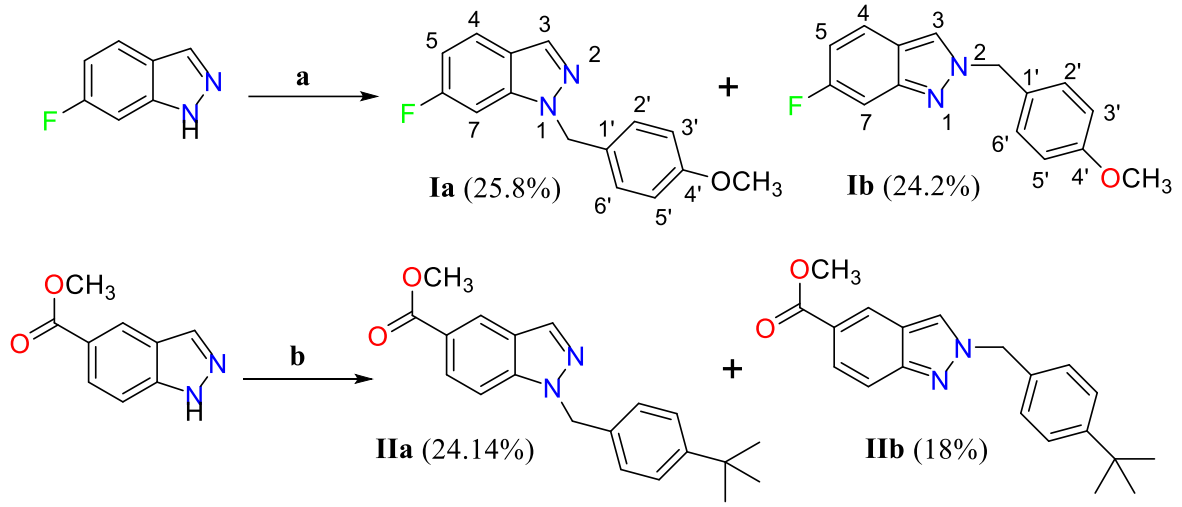
Purification (EtOAc : *n*-Hexane 12 : 100) first afforded the compound **IIa**, yield 0.078 g, 24.14%, mp : 130-132°C. **^{1}H-NMR** δ ppm ($CDCl_3$) : 1.29 (s, 9H, -C(C<u>*H*</u>$_3$)$_3$), 3.96 (s, 3H, -OC<u>*H*</u>$_3$), 5.60 (s, 2H, *N*-C<u>*H*</u>$_2$), 7.17 (d, 2H, *J* = 8.5 Hz, H-2',6'), 7.34 (d, 2H, *J* = 8.5 Hz, H-3',5'), 7.41 (d, 1H, *J* = 8.9 Hz, H-7), 8.04 (dd, 1H, *J* = 8.85 & 1.5 Hz, H-6), 8.16 (d, 1H, *J* = 0.85 Hz, H-3), 8.54 (s, 1H, H-4) ; **COSY** : [H-2',6' / H-3',5'], [H-6 / H-7] ; **NOESY** : [N-C<u>*H*</u>$_2$ /H-7], [N-C<u>*H*</u>$_2$ /H-2',6'], [-C(C<u>*H*</u>$_3$)$_3$ /H-3',5'] ; **^{13}C-NMR**, **HSQC** & **HMBC** δ ppm ($CDCl_3$) : 167.27 (C=O), 150.97 (C-4'), 141.3 (C-7a), 135.03 (C-4), 133.3 (C-1'), 127.2 (C-6), 127.02 (C-2',6'), 125.76 (C-3',5'), 124.7 (C-3), 124.05 (C-3a), 123.0 (C-5), 109.12 (C-7), 52.87 (*N*-<u>*C*</u>H$_2$), 52.09 (-OC<u>*H*</u>$_3$), 34.53 (-<u>*C*</u>(CH$_3$)$_3$, 31.27 (-C(<u>*C*</u>H$_3$)$_3$; **MS** (ESI+) m/z : 323 (M+H, 100%), 147 (M+H, 54%), $C_{20}H_{22}N_2O_2$.

Continued elution with (EtOAc : *n*-Hexane 15 : 100) provided **IIb**, yield 0.058 g, 18%, mp : 147-150°C. **^{1}H-NMR** δ ppm ($CDCl_3$) : 1.32 (s, 9H, -C(C<u>*H*</u>$_3$)$_3$), 3.94 (s,3H, -OC<u>*H*</u>$_3$), 5.59 (s, 2H, *N*-C<u>*H*</u>$_2$), 7.27 (d, 2H, *J* = 8.3 Hz, H-2',6'), 7.41 (d, 2H, *J* = 8.4 Hz, H-3',5'), 7.74 (d, 1H, *J* = 9.15 Hz, H-7), 7.91 (dd, 1H, *J* = 9.15 & 1.55 Hz, H-6), 8.04 (s, 1H, H-3), 8.54 (s, 1H, H-4) ; **COSY** : [H-2',6' / H-3',5'], [H-6 / H-7] ; **NOESY** : [N-C<u>*H*</u>$_2$ / H-3], [N-C<u>*H*</u>$_2$ / H-2',6'], [-C(C<u>*H*</u>$_3$)$_3$ / H-3',5'] ; **^{13}C-NMR**, **HSQC** & **HMBC** δ ppm ($CDCl_3$) : 167.5 (C=O), 151.76 (C-4'), 150.2 (C-7a), 132.1 (C-1'), 128 (C-2',6'), 126 (C-3',5'), 125.8 (C-6), 125.25 (C-3), 124.9 (C-4), 123.8 (C-3a), 121.4 (C-5), 117.4 (C-7), 57.54 (*N*-<u>*C*</u>H$_2$), 52.03 (-OC<u>*H*</u>$_3$), 34.64 (-<u>*C*</u>(CH$_3$)$_3$, 31.27 (-C(<u>*C*</u>H$_3$)$_3$; **MS** (ESI+) m/z : 323 (M+H, 100%), 147 (M+H, 74%), $C_{20}H_{22}N_2O_2$.

RESULT AND DISCUSSION

Targeted compounds were prepared using the methods outlined in Scheme 1. Alkylation of pyrazoles are region-specific, many times the mixtures of two isomers are obtained with same or different ratio [11]. In this study, when we attempted alkylation of indazoles with alkyl halides under basic conditions (K_2CO_3, in DMF), alkylation was formed N^1 and N^2 positions, so regiosomers **(Ia, IIa** as *N*1**)** and (**Ib, IIb** as *N*2) series were obtained.

In our experiments the occurring ratio of regioisomers were highly close to each other as expected (Scheme 1). While it was almost equal in the **I** series, the formation of **IIb** was occurred to a slightly lesser extent in the **II** series. One of the most decisive method 2D NOESY experiment has been used for the structure elucidation of the regioisomers firstly. Very strong NOE enhancements have been seen between *benzylic protons* and H-7 in the NOESY spectra of **Ia** and **IIa** (Figure 2). However, for the **b** series of the same compounds since there is no enough proximity between *N*-CH_2 and H-7, NOE enhancements were not observed as expected. In the spectra of **Ib** and **IIb** strong NOE interaction were seen between the *benzylic protons* and H-3 (Figure 2). These results were also supported by their HMBC correlations. It is possible to see the interactions of *benzylic* protons with C-7a in the HMBC spectra of **Ia** and **IIa** (Figure 3). In contrast, in the HMBC spectrum of **Ib** and **IIb** the interactions of *benzylic* protons with C-3 were seen (Figure 3).



Reagents: **a)** Anhydrous potassium carbonate / 4-methoxybenzyl chloride
b) Anhydrous potassium carbonate / 4-*tert*-butylbenzyl bromide

Scheme 1. Synthesis of new regioisomeric indazole analogues

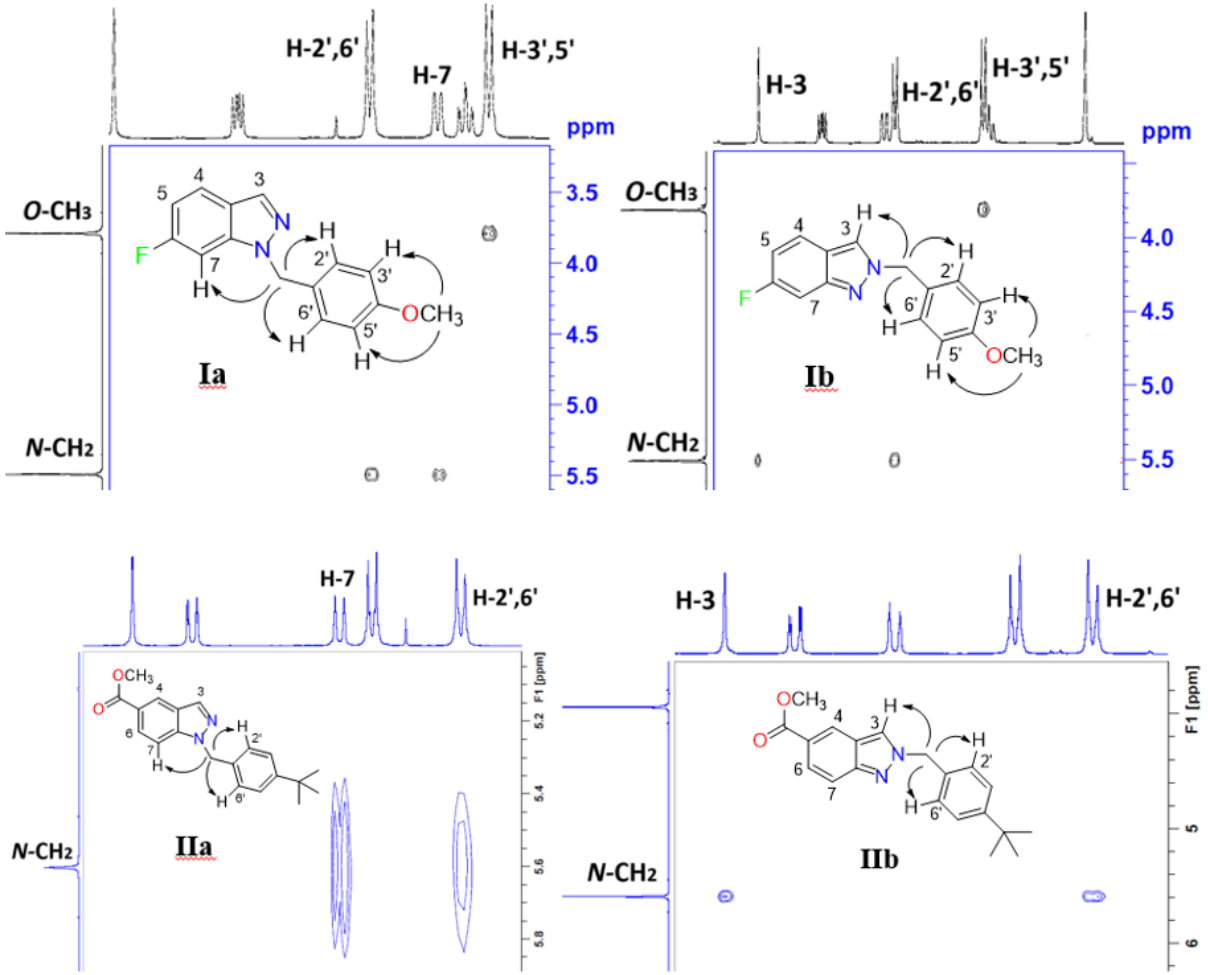


Figure 2. Partial NOESY spectra of compounds **Ia-b** and **IIa-b**

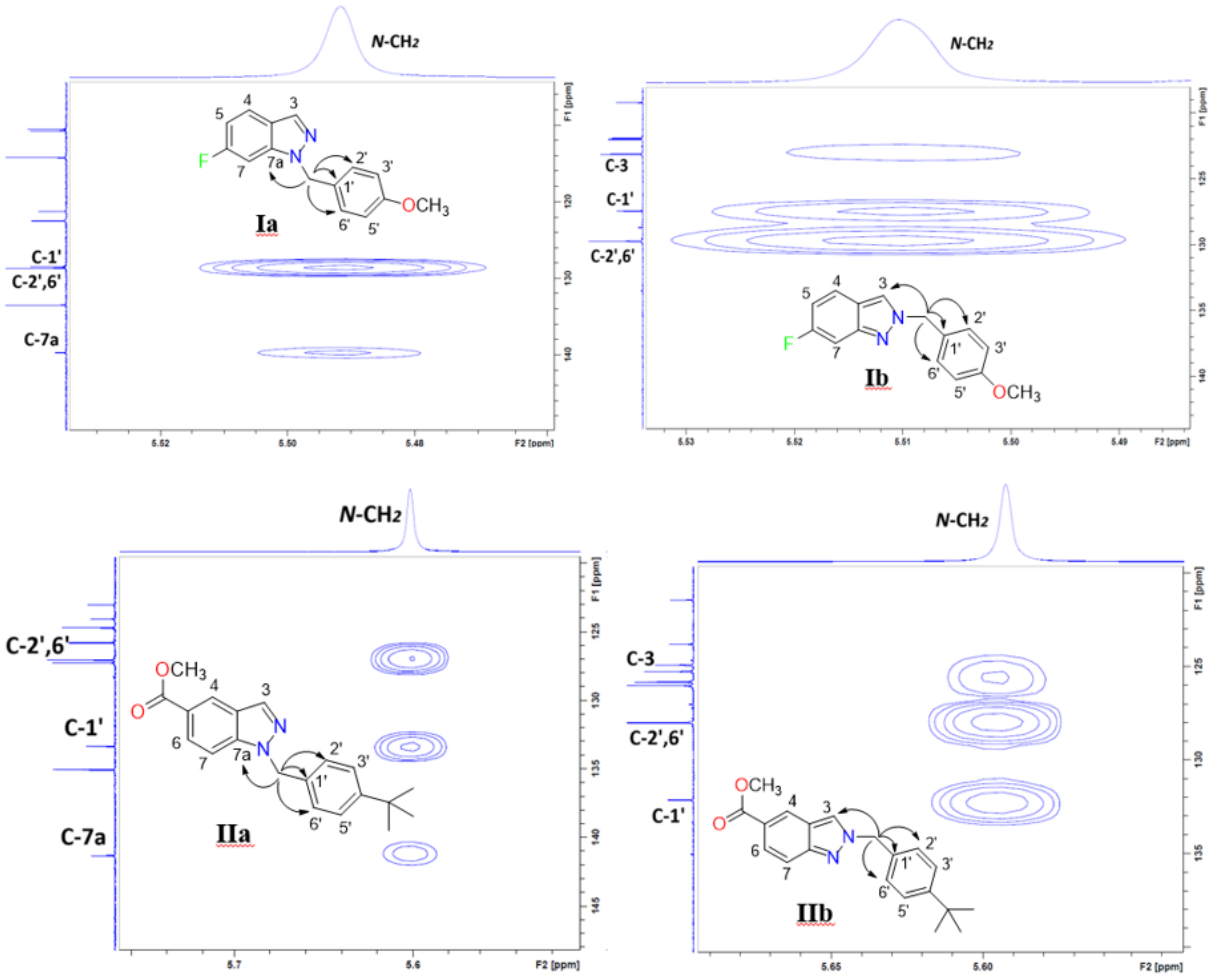


Figure 3. Partial HMBC spectra of compounds **Ia-b** and **IIa-b**

Conclusions

The alkylation of 1*H*- and 2*H* tautomeric forms of indazoles have been used to develop new active pharmaceutical ingredients. Hence their regioisomeric *N*-alkylation of these molecules would be of great importance to the pharmaceutical industry. It was found that *N*- alkylation of indazoles was realized with almost equal ratio (50%) in presence of anhydrous K_2CO_3 in DMF. NOESY and HMBC experiments were the decisive NMR techniquies for structural elucidation of these types of regioisomers. The complete structure elucidation of all synthesized compounds was performed using 1D and 2D NMR experiments including COSY, NOESY, gHSQC and gHMBC.

ACKNOWLEDGEMENTS

Central Laboratory of Pharmacy, Faculty of Ankara University provided support for acquisition of NMR and mass spectrometer used in this work.

AUTHOR CONTRIBUTIONS

Concept: H.G.; Design: F.D., H.G.; Control: F.D., H.G.; Sources: F.D., H.G.; Materials: F.D., H.G.; Data Collection and/or Processing: F.D., H.G.; Analysis and/or Interpretation: F.D., H.G.; Literature Review: F.D., H.G.; Manuscript Writing: H.G.; Critical Review: F.D., H.G.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Gaikwad, D.D., Chapolikar, A.D., Devkate, C.G., Warad, K.D., Tayade, A.P., Pawar, R.P., Domb, A.J. (2015) Synthesis of indazole motifs and their medicinal importance: An overview. European Journal of Medicinal Chemistry, 90, 707-731. [CrossRef]
2. Alam, R.M., Keating, J.J. (2021). Regioselective *N*-alkylation of the 1*H*-indazole scaffold; ring substituent and *N*-alkylating reagent effects on regioisomeric distribution. Beilstein Journal of Organic Chemistry. 17, 1939-1951. [CrossRef]
3. Uriarte, I., Reviriego, F., Calabrese, C., Elguero, J., Kisiel, Z., Alkorta, I.E., Cocinero, E.J. (2019). Bond length alternation observed experimentally: The case of 1*H*-indazole. European Journal of Medicinal Chemistry, 25, 10172. [CrossRef]
4. Hunt, K.W., Moreno, D.A., Suiter, N., Clark, C.T., Kim, G. (2009). Selective synthesis of 1-functionalized-alkyl-1*H*-indazoles. Organic Letters 11(21), 5054-5057. [CrossRef]
5. Alkorta, I., Elguero, J. (2005). Theoretical estimation of the annular tautomerism of indazoles. Journal of Physical Organic Chemistry, 18, 719-724. [CrossRef]
6. Doganc, F., Göker, H. (2024). Differentiation of regioisomeric *N*-alkylation of some indazoles and pyrazolopyridines by advanced NMR techniques. Magnetic Resonance in Chemistry, 62, 765-774. [CrossRef]
7. Doganc, F., Aydin, A.S., Şahin, E., Göker, H. (2020). Regioselective *N*-alkylation of some 2 or 6-chlorinated purine analogues. Journal of Molecular Structure, 1272, 134200. [CrossRef]
8. Puskullu, M.O., Doganc, F., Ozden, S., Sahin, E., Celik, I., Göker, H. (2021). Synthesis, NMR, X-ray crystallography and DFT studies of some regioisomers possessing imidazole heterocycles. Journal of Molecular Structure, 1243, 130811. [CrossRef]
9. Karaaslan, C., Doganc, F., Alp, M., Koc, A., Karabay, A.Z., Göker, H. (2020). Regioselective *N*-alkylation of some imidazole-containing heterocycles and their *in vitro* anticancer evaluation. Journal of Molecular Structure, 1205, 127673. [CrossRef]
10. Göker, H., Özden, S. (2019). Regioselective *N*-alkylation of 2-(3,4-dimethoxyphenyl)imidazo[4,5-*b*] and [4,5-*c*]pyridine oxide derivatives: Synthesis and structure elucidation by NMR. Journal of Molecular Structure, 1197, 183-195. [CrossRef]
11. Bulygina, L.A., Khrushcheva, N.S., Klemenkova, Z.S., Lyssenko, K.A., Peregudov, A.S., Solokov, V.I. (2018). Journal of Organometallic Chemistry, 867, 391-397. [CrossRef]