JJC *Jordan Journal of Chemistry* Vol. 10 No.3, 2015, pp. 179-186

Synthesis, Characterization and Evaluation of Antiinflammatory Activity of Some 1-(2,4-Dinitrophenyl) - 3-Aryl-7-(Substituted Benzylidene)-2,3,4,5,6,7-Hexahydro-1H-Indazoles

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Received on July 6, 2015 **Accepted on** Oct. 22, 2015

Abstract

The present work includes the synthesis of a series of new 1-(2,4-dinitrophenyl)-3-aryl-7-(substituted benzylidene)-2,3,4,5,6,7-hexahydro-1*H*-indazoles from substituted chalcones. These chalcones were prepared by the Claisen-Schmidt reaction of condensation of cyclohexanone with various substituted aromatic aldehydes. The synthesized compounds were confirmed by IR, ^{1}H NMR and MASS spectral analysis. The synthesized compounds were screened for anti-inflammatory activity by the gelatin zymography method. The synthesized compounds having unsubstituted phenyl ring at 3rd and 7th positions exhibited maximum activity.

Keywords: Indazoles; MMP-2 and MMP-9 Inhibitory activity; Gelatin zymography.

Introduction

Indazoles (benzpyrazoles) constitute an important class of heterocycles that display interesting biological properties such as anti-inflammatory, analgesic, antipyretic,[1,2] dopamine antagonistic,[3] anti-tumor,[4] antiemetic,[5] anticancer[6] and anti-HIV[7] activities. Among the heterocyclic compounds available for the preparation of potentially valuable new building-blocks in medicinal chemistry, the indazole nucleus is probably one of the least studied.

Inflammation is one of the hallmarks of cancer initiation and progression. It contributes to tumor initiation by inducing DNA damage and chromosomal instability as well as enhancing tumor cell proliferation. Inflammation also stimulates angiogenesis and tissue remodeling, which contribute to tumor cell invasion and metastasis.[8] Many studies have shown that chronic inflammation stimulates development and progression of cancer due to the release of matrix metalloproteinases (MMPs) from the

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inflammatory cells.[9] The MMPs function as essential regulators for the degradation of extracellular matrix (ECM) and basement membrane; thereby they contribute to the development and progression of human malignancies.[10] The MMP-9 (92 KDa) plays a crucial role in the mechanism of tumor invasion of many types of cancer.[11] Recent studies revealed that over-expression of MMP-9 in inflammation associated breast cancer,[12] colon cancer[13] and ovarian cancer[14] led to tumor metastasis. Thus, inhibition of MMP-9 activity could reduce inflammation and prevent cancer progression and metastasis as well.[15]

Hydrazine and its derivatives (N-N fragment) readily react with β-diketones, β-ketoesters, α,β-unsaturated carbonyl compounds and cyanoacetic esters to give indazoles, usually in good yields.[16] In this present study, the nitrogen containing fragment is 2,4-dinitrophenylhydrazine (N-N fragment hydrazine equivalent) and 2,6-bis-substituted benzylidene cyclohexanone (chalcone) serves as an excellent illustrative example in that it readily undergoes condensation reaction with 2,4-dinitrophenylhydrazine to produce the title compounds in good yield.

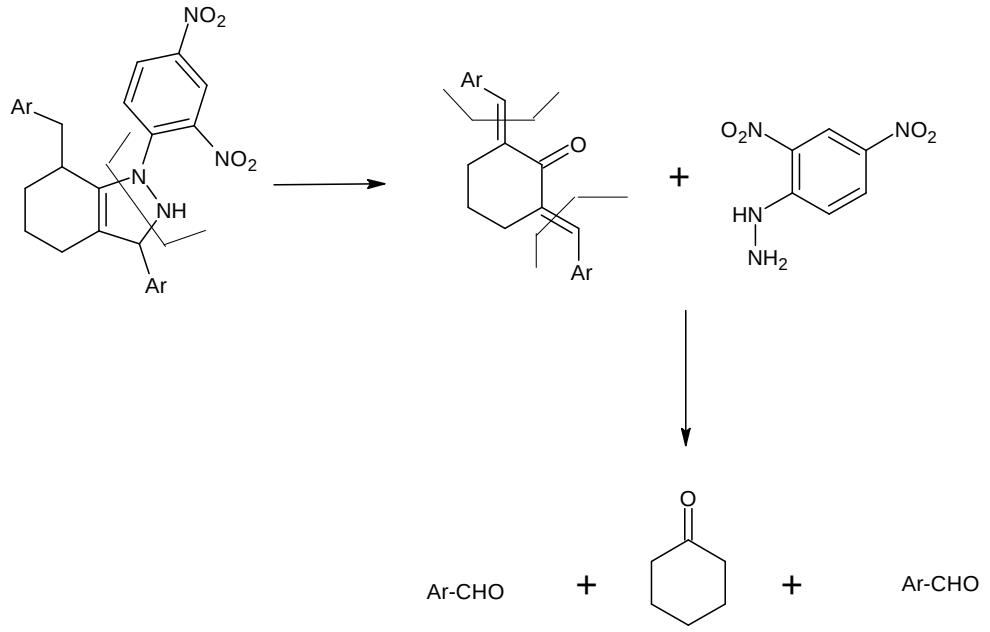
Recently our team reported the synthesis, characterization and evaluation of anticancer activity of some indazole derivatives.[17] In view of these observations and with an aim of continuing our previous work, 1-(2,4-dinitrophenyl)-3-aryl-7-(substituted benzylidene)-2,3,4,5,6,7-hexahydro-1*H*-indazoles were synthesized and screened for anti-inflammatory activity. Interestingly, all 6 compounds exhibited significant anti-inflammatory activity.

Materials and Methods

Melting points of the synthesized compounds were determined in an open capillary tube using digital melting point apparatus and are uncorrected. The purity of the synthesized compounds was established by thin layer chromatography using pre-coated silica gel strips, chloroform: acetone (2:1) as solvent system and UV-chamber for the detection of spots. Infrared spectra were recorded on a SHIMADZU FT-IR 4000 using KBr disks. Mass spectra were obtained on a JEOL GC mate II GC- Mass spectrometer at 70 eV using the direct insertion probe method. ^{1}H NMR spectra were taken on a BRUKER AV400-400MHz High resolution multinuclear FT-NMR spectrometer using TMS as internal standard and the solvent used was $CDCl_3$.

Retro-synthetic analysis (RSA)

Applying RSA on our target molecules by disconnecting the two C-N bonds of indazole resulted in α,β-unsaturated carbonyl compound (2,6-bis-substituted benzylidene cyclohexanone–chalcone) and 2,4-dinitrophenylhydrazine. Disconnection of isolated double bond in α,β-unsaturated carbonyl compounds resulted in aryl aldehydes and cyclohexanone (Scheme 1).



Scheme 1: RSA of 1-(2,4-dinitrophenyl)-3-aryl-7-(substituted benzylidene)-2,3,4,5,6,7-hexahydro-1*H*-indazoles (Y_{1-6}).

Experimental Work

Benzaldehyde, veratraldehyde, vanillin, ethylvanillin, anisaldehyde, 4-dimethyl-aminobenzaldehyde, cyclohexanone,2,4 dinitrophenylhydrazine, ethanol, pyridine and sodium hydroxide were used to synthesize six 1-(2,4-dinitrophenyl)-3-aryl-7-(substituted benzylidene)-2,3,4,5,6,7-hexahydro-1*H*-indazoles.

The synthesis of various 1-(2,4-dinitrophenyl)-3-aryl-7-(substituted benzylidene)-2,3,4,5,6,7-hexahydro-1*H*-indazoles starts with the reaction of cyclohexanone and substituted aromatic aldehydes via the Claisen-Schmidt reaction to yield 2,6-bis substituted benzylidenecyclohexanones (chalcones-C_{1-6}). These synthesized 2,6-bis substituted benzylidenecyclohexanones were refluxed with 2,4-dinitrophenylhydrazine in the presence of pyridine to get 1-(2,4-dinitrophenyl)-3-aryl-7-(substituted benzylidene)-2,3,4,5,6,7-hexahydro-1*H*-indazoles (Y_{1-6}).

General synthesis of 2,6-bis(substituted benzylidene)cyclohexanones (C1-6)

To a mixture of cyclohexanone (1mL, 0.01 mol) and substituted aromatic aldehydes (0.02 mol) in ethanol (50mL) cooled at 5-10^{0}C, aqueous sodium hydroxide solution (70%, 5 mL) was added dropwise with constant stirring. The reaction mixture was further stirred for 2 hrs and left overnight and neutralized with concentrated hydrochloric acid, the separated solid was then filtered, washed with cold water until the washings are neutral to litmus and recrystallized from hot water.

General synthesis of 1-(2,4-dinitrophenyl)-3-aryl-7-(substituted benzylidene)-2,3,4,5,6, 7-hexahydro-1H-indazoles (Y1-6).

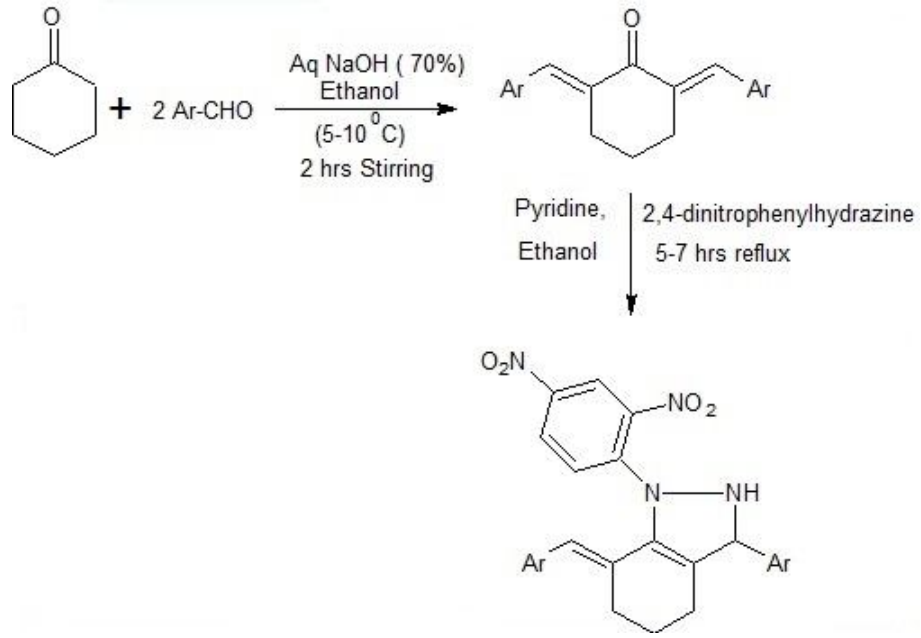
A mixture of 2,6-bis substituted benzylidenecyclohexanone (0.01 mol) and 2,4-dinitrophenylhydrazine (1.98 g, 0.012 mol) were dissolved in ethanol, the reaction mixture was refluxed for 5-7 hours in the presence of pyridine. The reaction mixture was poured into crushed ice with constant stirring and then filtered. The crude product is recrystallized with ethanol. The purity of the compound was established by TLC using a mixture of chloroform and acetone (2:1) as mobile phase.

Anti-inflammatory activity (Gelatin zymography method)

SDS-PAGE (Sodium dodecylsulfate-Polyacrylamide gel electrophoresis) was carried out according to the gelatin zymography protocol.[18] Zymogram gel consisting of 7.5% polyacrylamide gel copolymerized with gelatin (1mg/mL) was prepared for electrophoresis. Following electrophoresis, the gel was washed successively with 50 mL of 2.5% (v/v) TritonX-100 in distilled water for an hour to remove SDS. The gel was then incubated with developing solution ($CaCl_2$ 10 mM, Triton X-100 1%, and Tris buffer, 50 mM, pH 7.4) at 32°C for 18 h. Further, the gel was stained with Coomassie brilliant blue R250 for 2 h and destained overnight to reveal the bands. The bands on gel reflect the MMP-2 and MMP-9 inhibitory effects of title compounds.

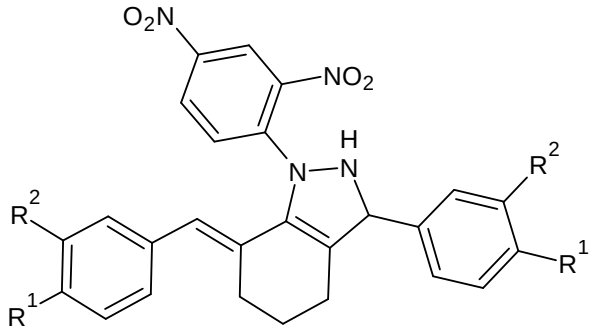
Results and Discussion

The title compounds were synthesized according to Scheme 2, the physicochemical characterization and structural confirmation (IR, NMR and MS) are presented in Table 1 and Table 2. All synthesized compounds were obtained as crystalline needles with sharp melting points. The yields of the product were found to be satisfactory. All compounds were in conformity with the structures envisaged.



Scheme 2: Synthesis of title compounds.

Table 1: Physicochemical properties of Title Compounds (Y1-6).



Compound Code	Molecular formula	R^1	R^2	Molecular Weight	R_f value	Melting Point (^{0}C)	Percentage Yield (%)
Y-1	$C_{26}H_{22}N_4O_4$	-H	-H	454.48	0.63	145	85
Y-2	$C_{30}H_{30}N_4O_8$	$-OCH_3$	$-OCH_3$	574.58	0.57	192	82
Y-3	$C_{28}H_{26}N_4O_8$	-OH	$-OCH_3$	546.53	0.70	172	70
Y-4	$C_{30}H_{30}N_4O_8$	-OH	$-OC_2H_5$	574.58	0.68	185	72
Y-5	$C_{28}H_{26}N_4O_6$	$-OCH_3$	-H	514.53	0.73	153	84
Y-6	$C_{30}H_{32}N_6O_4$	$-N(CH_3)_2$	-H	540.61	0.65	164	80

Table 2: Spectral details and IUPAC names of title compounds (Y1-6).

7-benzylidene-1-(2,4-dinitrophenyl)-3-phenyl-2,3,4,5,6,7-hexahydro-1*H*-indazole (Y1):
IR (KBr):1084 (C-N), 1304 (N-Hdef), 3345 (N-Hstr), 1526(NO_2), 1332(C-N)cm^{-1}. ^{1}HNMR($CDCl_3$): δ 1.35 (m,4H,$2CH_2$), 2.0 (m,2H,CH_2), 4.7 (d,1H,CH), 6.5 (m,10H, Aromatic), 8.5 (m,3H,Aromatic), 9.5 (s,1H,NH) ppm. MS(m/z): 454.60 (M^+).

7-(3,4-dimethoxybenzylidene)-3-(3,4-dimethoxyphenyl)-1-(2,4-dinitrophenyl)-2, 3, 4, 5, 6, 7-hexahydro-1*H*-indazole (Y2):
IR (KBr): 1069 (C-N), 1285 (N-Hdef), 3308 (N-Hstr), 1531 (NO_2), 1040 (C-O-C), 1309 (C-N) cm^{-1}. ^{1}HNMR($CDCl_3$): δ 1.42 (m,4H,$2CH_2$), 2.1 (m,2H,CH_2), 4.9 (d,1H,CH), 3.5 (s,12H,OCH_3), 8.2 (m,3H,Aromatic), 7.5 (m,6H,Aromatic), 9.0 (s,1H,NH) ppm. MS(m/z): 575.91 (M^+).

1-(2,4-dinitrophenyl)-7-(4-hydroxy-3-methoxybenzylidene)-3-(4-hydroxy-3-methoxyphenyl) - 2, 3,4,5,6,7-hexahydro-1*H*-indazole (Y3):
IR (KBr): 1059 (C-N), 1269 (N-Hdef), 3302 (N-Hstr), 1021 (C-O-C), 1547 (NO_2), 1318 (C-N), 3552 (OH) cm^{-1}. ^{1}HNMR ($CDCl_3$): δ 1.42 (m,4H,$2CH_2$), 2.1 (m,2H,CH_2), 4.9 (d,1H,CH), 3.74 (s,6H,OCH_3), 5.35 (s,2H,OH), 8.45 (m,3H,Aromatic), 7.12 (m,6H,Aromatic), 9.2 (s,1H,NH)ppm. MS(m/z): 547.13 (M+)

1-(2,4-dinitrophenyl)-7-(4-hydroxy-3-ethoxybenzylidene)-3-(4-hydroxy-3-ethoxyphenyl)-2,3,4,5,6,7-hexahydro-1*H*-indazole (Y4):
IR (KBr): 1056 (C-N), 1278 (N-Hdef), 3367 (N-Hstr), 1017 (C-O-C), 1542 (NO_2), 1326 (C-N), 3616 (OH) cm^{-1}. ^{1}HNMR ($CDCl_3$): δ 1.42 (m,4H,$2CH_2$), 2.1 (m,2H,CH_2), 4.9 (d,1H,CH), 0.9 (m,6H,CH_3), 1.3 (m,4H,OCH_2), 5.35 (s,2H,OH), 8.45 (m,3H,Aromatic), 7.2 (m,6H,Aromatic), 9.0 (s,1H,NH) ppm. MS(m/z): 575.49 (M+).

1-(2,4-dinitrophenyl)-7-(4-methoxybenzylidene)-3-(4-methoxyphenyl)-2,3,4,5,6,7-hexahydro-1*H*-indazole (Y5):
IR (KBr): 1066 (C-N), 1281 (N-Hdef), 3315 (N-Hstr), 1529 (NO_2), 1041 (C-O-C), 1317 (C-N) cm^{-1}. ^{1}HNMR ($CDCl_3$): δ 1.42 (m,4H,$2CH_2$), 2.1 (m,2H,CH_2), 4.9 (d,1H,CH), 3.3 (s,6H,OCH_3), 8.4 (m,3H,Aromatic), 7.71 (m,8H,Aromatic), 9.1 (s,1H,NH) ppm. MS(m/z):515.23(M^+).

7-(4-dimethylaminobenzylidene)-3-(4-dimethylaminophenyl)-1-(2,4-dinitrophenyl)-2,3,4,5,6,7-hexahydro-1*H*-indazole (Y6):
IR (KBr): 1084 (C-N), 1304 (N-Hdef), 3286 (N-Hstr), 1576 (NO_2), 2985 (CH_3), 1172 (ArC-N-C) cm^{-1}. ^{1}HNMR($CDCl_3$): δ 0.9 (m,12H,CH_3), 1.52 (m,4H,$2CH_2$), 2.0 (m,2H,CH_2), 4.6 (d,1H,CH), 8.8 (m,3H,Aromatic), 7.5 (m,8H,Aromatic), 9.3 (s,1H,NH) ppm. MS(m/z): 541.79(M^+).

All synthesized indazoles were evaluated for anti-inflammatory activity (MMP-2 and MMP-9 inhibitory activity) in the gelatin zymography method using isolated tonsil tissue. Matrix metalloproteinases (MMPs), in particular, the MMP-9, are essential regulators of extracellular matrix (ECM) and recruit the inflammatory cells during chronic inflammation which involves a series of complex morphological changes in cell barrier, cell-cell interaction and cell matrix interaction. In this study, we assessed the ability of the title compounds as anti-inflammatory agents to inhibit MMP-9 activity. Gelatin zymography clearly showed that the title compounds inhibit the MMP-2 and MMP-9 activity. However, further molecular studies are required to understand their anti-inflammatory mechanism.

All synthesized compounds (Y1-6) were found to exhibit anti-inflammatory activity. From Table 3, it is clear that compound (Y1) was found to be the most potent. Compound (Y6) was found to be slightly less potent than (Y1), followed by the compounds Y5, Y3, Y4 and Y2, respectively. Anti-inflammatory activity of synthesized compounds in the order of their increasing potency are as follows: Compound (Y1)>(Y6)>(Y5)>(Y3)>(Y4)>(Y2).

Table 3: Results of anti-inflammatory activity (gelatin zymography) of title compounds (Y1-6).

Sl.No.	Compound code	Percentage of activity
1.	Y_1	80%
2.	Y_2	50%
3.	Y_3	60%
4.	Y_4	55%
5.	Y_5	70%
6.	Y_6	75%

Compound (Y1) possesses a benzene ring at the 3rd position and an unsubstituted benzylidine group at the 7th position of the hexahydroindazole nucleus. Compound (Y6) possesses 4-dimethylaminophenyl ring at the 3rd position and 4-dimethylaminobenzylidine group at the 7th position of the hexahydroindazole nucleus. Compounds (Y5, Y3, Y4 and Y2) possess alkoxy and hydroxyl groups on aromatic rings.

The compounds having electron donating groups in the benzene ring located at the 3rd and 7th positions of hexahydroindazole have remarkably less activity than the remaining compounds. From these results, it has been revealed that the compound should have an unsubstituted benzene ring at the 3rd and 7th positions of the hexahydroindazole nucleus for maximal activity. Mono and disubstitution on benzene ring located at the 3rd and 7th positions of hexahydroindazole seems unfavorable for anti-inflammatory activity.

Conclusion

From the present investigation, it is concluded that we have successfully synthesized the title compounds and evaluated their anti-inflammatory activity. 1-(2,4-dinitrophenyl)-3-aryl-7-(substituted benzylidene)-2,3,4,5,6,7-hexahydro-1*H*-indazoles showed strong anti-inflammatory activity by suppressing the expression of MMP-9 in the gelatin zymography method. These findings provide evidence that the anti-inflammatory potential of title compounds could be useful in the development of new therapeutic strategies for inflammation associated cancer.

The results revealed that the synthesized compound (Y1) possessing a benzene ring at the 3rd position and an unsubstituted benzylidine group at the 7th position of the hexahydroindazole nucleus exhibited good anti-inflammatory activity. The study revealed the necessity of synthesizing many more compounds with other substituents including electron withdrawing groups at the 4th position of the benzene ring located at the 3rd and 7th positions of indazole. Such compounds may emerge as much more potent anti-inflammatory agents.

However, further studies are required to establish the mechanism of action of title compounds. The contributing physico-chemical properties for the anti-inflammatory activity need to be established by detailed QSAR studies, which may provide insights into the structural requirements of this class of molecules.

Acknowledgement

The authors are thankful to the authorities and management of University of Gondar and Sri Padmavathi school of Pharmacy for providing facilities to carry out this research work.

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