Design, synthesis and characterization of 1 *H*-pyridin-4-yl-3,5-disubstituted indazoles and their anti-inflammatory and analgesic activity

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Abstract. A new series of 1*H*-pyridin-4-yl-indazole-3-carboxylic acid and its derivatives were synthesized from indazole-3-carboxylic acid methyl ester and 2-cyano-4-chloropyridine. All the new compounds have been characterized by spectral data and subsequently evaluated for their anti-inflammatory and analgesic activity.

Keywords. 1*H*-pyridin-4-yl-3,5-disubstituted indazoles; anti-inflammatory and analgesic activity.

1. Introduction

Among the heterocyclic compounds, indazole moiety has been of great interest to the research community due to its wide variety of biological properties such as anti-cancer,¹ anti-hypertensive,² anti-viral³ and anti-angiogenic agents.⁴ Moreover, presently marketed anti-inflammatory drugs, namely Bendazac and Benzydamine are having indazole moiety (figure 1). Last decade (2001–2010) has witnessed an unprecedented explosion of research on the diverse biological properties of compounds having an indazole moiety.

As part of our ongoing research on indazoles⁵ and pyridines,⁶ we were interested to study 1*H*-(4-pyridyl) indazoles, because pyridine moiety alone has wide biological properties.⁷ Indazoles with pyridine substituted compounds also have anti-cancer properties.⁸ Based on this concept, we thought that by incorporating 2-cyanopyridine at N1-position of indazole and further conversions may lead to superior anti-inflammatory and analgesic activity than the existing ones.

Generally, the non-steroidal inflammatory drugs are prescribed for the treatment of pain, fever, acute and chronic inflammation. There is a continuous effort to discover new and better anti-inflammatory drugs.

2. Experimental

2.1 General information

All reagents were obtained commercially and were of the highest commercial quality and used without further purification. Solvents were freshly distilled and used. Melting points were determined in open capillaries and are uncorrected. TLC or HPLC are routinely used to check the purity of all compounds. IR spectra were recorded on a Perkin-Elmer model 2000 instrument in KBr phase. ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were recorded in CDCl₃ or DMSO using Bruker instrument and Mass spectra were recorded on a Perkin-Elmer mass spectrometer operating at 70 eV.

2.2 General procedure for the preparation of 1-(2-cyanopyridin-4-yl)-1*H*-indazole-3-carboxylic acids **3a–b**

To a solution of 2-cyano-4-chloropyridine **2** (50 g, 0.361 mol), methyl 1*H*-indazole-3-carboxylate **1a** (63.5 g, 0.361 mol) in DMSO (300 mL), CuI (6.87 g, 0.036 mol), *L*-proline (8.3 g, 0.0722 mol) and cesium carbonate (235.2 g, 0.722 mol) were added at RT. Reaction mass was stirred for 6–12 h at 75–80°C. After completion of reaction, the reaction mass was quenched in water (2 L) and stirred for 30 min. The undissolved substances were filtered through celite pad. The filtrate pH was adjusted to 2.0–2.5 with Conc.HCl to get the crude product

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Figure 1. Indazole based anti-inflammatory and analgesic drugs.

(58 g). This crude product was recrystallized from ethyl acetate to get the pure product (55 g).

2.2a 1-(2-cyano-pyridin-4-yl)-1H-indazole-3-carboxylic acid (3a): Yield: (57.7%); MR: 242–245°C; IR(KBr, cm^{-1}): 1704 (C=O), 2240(-CN); ^1H -NMR (400 MHz, DMSO): δ 7.47 (t, 1H, $J = 7.59$ Hz), 7.63 (t, 1H, $J = 7.78$ Hz), 8.10 (m, 3H), 8.52 (d, 1H, $J = 1.24$ Hz), 8.88 (d, 1H, $J = 5.44$ Hz), 13.73 (s, 1H); ^{13}C -NMR (DMSO, 100 MHz): δ 112.5, 117.4, 119.4, 120.9, 122.7, 124.9, 125.0, 129.3, 134.5, 139.7, 140.2, 146.8, 153.1, 163.0; ms: m/z 265 (264) (M^+). Anal. Calcd. for $\text{C}_{14}\text{H}_8\text{N}_4\text{O}_2$: C, 63.64; H, 3.05, N, 21.20, Found: C, 63.80; H, 3.30, N, 21.39.

2.3 General procedure for the preparation of 1-(2-cyanopyridin-4-yl)-1H-indazole-3-carboxamides 4(a–g)

To a solution of 1-(2-cyanopyridin-4-yl)-1H-indazole-3-carboxylic acid **3a** (10 g, 0.037 mol), DMF (0.1 mL) in ethylene dichloride (100 mL), thionyl chloride (9 g, 0.075 mol) was added at 40–45°C and maintained for 1 h at 80–85°C. Upon completion of the reaction, the solvent was distilled off completely under atmospheric pressure to get the acid chloride. Fresh ethylene dichloride (50 mL) was added to the acid chloride, and cooled to 15°C and diethyl amine (8 g, 0.111 mol) was added at 15–30°C to pH 8.0–8.5 and stirred for 30 min. To the reaction mass water (50 mL) was added and the organic layer was separated and washed with water (10 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to get the product.

2.3a 1-(2-Cyano-pyridin-4-yl)-1H-indazole-3-carboxylic acid diethylamide (4a): Yield: (80%); MR: 82–84°C; IR(KBr, cm^{-1}): 1630 (C=O), 2235 (-CN); ^1H -NMR (CDCl_3 , 400 MHz): δ 1.33 (m, 6H), 3.61 (q, 2H), 3.70 (q, 2H), 7.37 (t, 1H, $J = 7.56$ Hz), 7.57 (t, 1H, $J = 7.72$ Hz), 7.86 (d, 1H, $J = 8.80$ Hz), 7.96 (dd, 1H, $J_1 = 1.84$, $J_2 = 5.46$ Hz), 8.16 (s, 1H), 8.18 (s, 1H),

8.77 (d, 1H, $J = 5.60$ Hz); ^{13}C -NMR (100 MHz, CDCl_3): δ 12.8, 14.7, 40.8, 43.3, 110.4, 116.8, 116.9, 119.4, 123.5, 124.1, 126.3, 129.2, 135.1, 138.7, 144.0, 147.4, 152.3, 161.8; ms: m/z 320 (319) (M^+). Anal. Calcd. for $\text{C}_{18}\text{H}_{17}\text{N}_5\text{O}$: C, 67.70; H, 5.37; N, 21.93, Found: C, 67.93; H, 5.47; N, 22.10.

2.4 General procedure for the preparation of 1-(2-carbamoylpyridin-4-yl)-1H-indazole-3-carboxamides 5(a–g)

To a solution of Conc. H_2SO_4 (36 mL) and water (0.32 mL, 0.018 mol), sodium chloride (0.1 g, 0.0017 mol) was added at 10–15°C over a period of 30 min, and was heated to 35°C. 1-(2-cyano-pyridin-4-yl)-1H-indazole-3-carboxylic acid diethylamide **4a** (6 g 0.018 mol) was added at 35–40°C (by maintaining the temperature with external cooling) over a period of 30 min. The reaction mixture was stirred for 1 h at 40–45°C, and after completion of the reaction, the mass was quenched into ice-water (250 mL) and stirred for 30 min at 10–15°C. The precipitated product is filtered and washed with chilled DM-Water (2×100 mL) to yield the desired product.

2.4a 1-(2-carbamoyl-pyridin-4-yl)-1H-indazole-3-carboxylic acid diethylamide (5a): Yield: (80%); MR: 132–134°C; IR(KBr, cm^{-1}): 1695 (C=O), 1661 (C=O), 3376 (-NH); ^1H -NMR (DMSO, 400 MHz): δ 1.14–1.25 (m, 6H), 3.52 (q, 2H), 3.63 (q, 2H), 7.40 (t, 1H, $J = 7.39$ Hz), 7.64 (t, 1H, $J = 7.56$ Hz), 7.84 (s, 1H), 8.02 (d, 1H, $J = 7.97$ Hz), 8.08 (d, 2H, $J = 7.51$ Hz), 8.25 (s, 1H), 8.46 (s, 1H), 8.78 (d, 1H, $J = 5.15$ Hz); ^{13}C -NMR (DMSO, 100 MHz): δ 13.1, 15.0, 39.6, 43.2, 111.8, 113.2, 117.2, 122.8, 124.0, 125.5, 129.3, 138.8, 143.0, 147.4, 150.7, 152.6, 161.8, 165.7; ms: m/z 338 (337) (M^+). Anal. Calcd. for $\text{C}_{18}\text{H}_{19}\text{N}_5\text{O}_2$: C, 64.08; H, 5.68; N, 20.76, Found: C, 64.29; H, 5.58; N, 20.96.

2.5 General procedure for the preparation of 1-(2-methylcarbamoyl-pyridin-4-yl)-1H-indazole-3-carboxamides 6(a–g)

To a solution of **4a** (5 g, 0.015 mol) in methanol (100 mL) was added thionyl chloride (7.5 g, 0.063 mol) at 40–45°C over a period of 10–15 min. The reaction mixture was stirred for 1 h at 50–55°C. The solvent was distilled off to get the crude product. Fresh methanol (50 mL) was added to the residue and mono methylamine gas was bubbled at 10–40°C over a period of 20 min. The reaction mass was stirred for 30 min at

25–30°C, distilled off the solvent to get the crude. Water (10 mL) and dichloromethane (20 mL) were added and stirred for 10 min. The organic layer was separated and concentrated completely to get the **6a**, which was slurred in hexane (10 mL) and filtered to get the desired pure product.

2.5a 1-(2-methylcarbamoyl-pyridin-4-yl)-1*H*-indazole-3-carboxylic acid diethylamide (6a**):** Yield (55%); MR:132–134°C; IR (KBr, cm^{-1}): 1669 (C=O), 1613 (C=O) and 3358 (–NHCH₃); ¹H (400 MHz,

CDCl₃): δ 1.18 (t, 3H, J = 7.16 Hz), 1.26 (t, 3H, J = 7.02 Hz), 3.02 (d, 3H, J = 5.01 Hz), 3.58 (q, 2H), 3.71 (q, 2H), 7.30 (t, 1H, J = 7.51 Hz), 7.48 (t, 1H, J = 7.70 Hz), 7.83 (dd, 1H, J_1 = 5.30, J_2 = 1.84 Hz), 7.91 (d, 1H, J = 8.48 Hz), 7.93 (s, 1H), 8.17 (d, 1H, J = 8.04 Hz), 8.57 (d, 1H, J = 5.44 Hz), 8.62 (d, 1H, J = 1.68 Hz); ¹³C-NMR (100 MHz, CDCl₃): δ 12.8, 14.6, 26.0, 40.8, 43.4, 40.8, 112.8, 116.9, 123.3, 123.6, 126.1, 128.6, 138.9, 142.9, 147.9, 149.4, 151.7, 162.2, 164.1. ms: m/z 352 (351) (M⁺). Anal. Calcd. for C₁₉H₂₁N₅O₂: C, 64.94; H, 6.02; N, 19.93, Found: C, 65.14; H, 6.20; N, 20.10.

Scheme 1. Synthesis of 1*H*-pyridin-4-yl-indazole-3-carboxylic acids and derivatives.

Table 1. Analgesic activity of novel synthetic compounds (10 mg/kg) and indomethacin (10 mg/kg).

Compound	Mean Response (sec)	
	30 min	60 min
Control	2.50±0.22	2.66±0.21
indomethacin	5.50±0.22**	5.66±0.21**
3a	5.33±0.49**	5.50±0.34**
4a	7.16±0.30**	8.16±0.30**
4b	5.16±0.30**	5.50±0.34**
4f	5.16±0.30**	3.16±0.30
4d	2.83±0.30	2.66±0.33
4g	3.16±0.47	3.16±0.40
5a	2.83±0.30	2.83±0.16
5d	5.16±0.30**	5.50±0.42**
5e	2.83±0.30	2.83±0.30

Data represent mean ± SE(n=6).

Data were analyzed by using one-way ANOVA followed by Dunnett's test.

** P<0.01 when compared with control group.

The active compounds are marked in bold letters.

2.6 Pharmacological evaluation of newly synthesized compounds

All compounds synthesized and reported in this article were screened for their pharmacological activities such as *in-vivo* analgesic and anti-inflammatory activities. These activities were carried out by measuring the physiological responses of animals to the thermal and chemical stimuli. For analgesic activity, tail immersion method⁹ in rats at 10 mg/kg body weight (b.w.) was performed. The percentage increase in mean response time was calculated and compared with indomethacin. For anti-inflammatory activity, carragenan induced

inflammation on hind paw edema method¹⁰ at 10 mg/kg b.w was performed. The percent of inhibition was determined for synthesized compounds as well as standard drug indomethacin.

2.6a Anti-inflammatory activity: The anti-inflammatory activity of the synthesized compounds are assessed by the carragenan induced inflammation on hind paw edema method. In all the groups vehicle, indomethacin (10 mg/kg, po) or test compounds (10 mg/kg, po) were administered 30 min prior to induction of edema. The edema of hind paw was induced by injection of 0.1 mL of 1% carragenan solution into sub-plantar region of right hind paw. The volume of paw was measured plethysmographically 30, 60, 120 and 180 min, after the injection of the irritant. Percent inhibition of the edema between control group and the test groups were calculated and compared with the group received standard drug indomethacin (10 mg/kg, po).

2.6b Analgesic activity: The analgesic activity of the synthesized compounds are assessed by the tail immersion method. The lower portion of the tail was immersed in a water bath at 55 ± 0.5°C. Vehicle (1% CMC) was given to the control group; while the reference group was given indomethacin (10 mg/kg, po) and the other groups were treated with newly synthesized compounds (10 mg/kg, po). The time in seconds for the tail withdrawal from the water was taken as the reaction time, with a cut-off time of 10 sec. The reaction time for the various groups was determined at 30 min and 60 min, after the drug treatment.

Table 2. Anti-inflammatory activity of novel synthetic compounds (10 mg/kg) and indomethacin (10 mg/kg).

Compound	Paw edema							
	30 min	% edema inhibition	60 min	% edema inhibition	120 min	% edema inhibition	180 min	% edema inhibition
Control	2.15±0.04	—	2.33±0.06	—	2.49±0.03		2.79±0.04	
indomethacin	1.28±0.04	40.46**	1.45±0.06	37.76**	1.52±0.02	38.95**	1.71±0.12	38.70**
3a	1.46±0.06	32.09**	1.36±0.06	41.63**	2.42±0.03	2.81	2.61±0.05	6.45
4a	1.95±0.05	9.30	2.21±0.05	5.15	2.41±0.06	3.21	2.52±0.12	9.67
4b	1.30±0.03	39.53**	1.55±0.05	33.47**	1.51±0.02	39.35**	1.69±0.06	39.42**
4f	1.32±0.03	38.60**	1.48±0.06	36.48**	1.49±0.04	40.16**	1.71±0.12	38.70**
4d	1.98±0.04	7.90	2.08±0.06	10.72	2.42±0.13	2.81	2.63±0.21	5.73
4g	1.95±0.06	9.30	2.16±0.06	7.29	1.61±0.05	35.34**	1.69±0.08	39.42**
5a	2.01±0.06	6.51	2.13±0.06	8.58	2.35±0.12	5.62	2.73±0.11	2.15
5d	1.38±0.04	35.81**	1.93±0.10	17.16**	2.13±0.11	14.45	2.63±0.12	5.73
5e	1.96±0.04	8.83	2.15±0.09	7.72	2.11±0.13	15.26	2.35±0.21	15.77

Data represent mean ± SE (n=6).

Data were analyzed using one-way ANOVA followed by Dunnett's test.

** P<0.01 when compared with control group and the active compounds are marked in bold letters.

3. Results and Discussion

3.1 Chemistry

We herein report a general and novel synthesis of 1*H*-pyridin-4-yl-indazole-3-carboxylic acid and its derivatives in good yields. Condensation of 1*H*-indazole-3-carboxylic acid methyl ester **1a–b** with 2-cyano-4-chloropyridine **2** in the presence of CuI,¹¹ *L*-proline and cesium carbonate gave 1*H*-pyridin-4-yl-indazole-3-carboxylic acids **3a–b** in moderate to good yields. Later the compounds **3(a–b)** are converted to acid chloride and reacted with individual amines (**R**₂) to give 1-(2-cyanopyridin-4-yl)-*N, N*-diethyl-1*H*-indazole-3-carbox-amides **4(a–g)** with good yields and **4g** was confirmed by single crystal structure analysis¹² (CCDC 947891). Later, hydrolysis of **4(a–g)** under acidic conditions gave 1-(2-carbamoylpyridin-4-yl)-*N, N*-diethyl-1*H*-indazole-3-carboxamides **5(a–g)**, and also **4(a–b)** were converted into picolinates in the presence of methanol and thionyl chloride and then reacted with mono methylamine in methanol to give corresponding 1-(2-methylcarbamoyl-pyridin-4-yl)-1*H*-indazole-3-carboxylic acid amides (**6a–b**). The total reaction strategy is depicted in scheme 1.

3.2 Structure activity relationship (SAR)

The analgesic activity of the novel compounds were analyzed by the tail immersion method.⁹ As part of SAR studies, we initially screened the analgesic activity on compound **3a**. Oral treatment with compound **3a** with –CN substitution at 2nd position of pyridine moiety as well as –COOH substitution at C-3 position of indazole moiety showed significant analgesic activity and this effect was similar to that of indomethacin. We prepared a series of C-3&C-5 derivatives based on this result.

Converting –COOH group into carboxamides with pyrrolidine (**4b**) and *N*-ethyl piperazine (**4f**) showed equipotent activity. Whereas, the compound **4d** morpholine amide showed poor activity. Furthermore, the compound **4a** with diethyl amide showed a remarkable increase in potency. The compound **4g** with bromo substitution at C-5 position showed moderate activity.

Based on the above results, we focused on modification at 2nd position of pyridine ring. Replacement of –CN substitution by –CONH₂ (**5a**) showed moderate activity and similar effect is observed with compound **5e**. The compound **5d** showed equipotent activity when compared to reference standard indomethacin (table 1).

As part of other bio studies, we were interested to check the anti-inflammatory activity of all these compounds. The compound **3a** with –CN substitution at 2nd position of pyridine ring and –COOH substitution at C-3 position showed significant effect at 30 and 60 min but failed to show similar effect at 120 and 180 min. Furthermore, the compound **4b** with pyrrolidine substitution and compound **4f** with *N*-ethyl piperazine showed a remarkable activity in all intervals and this effect is similar to that of indomethacin. Treatment with compound **4g** showed equipotent activity at 120 and 180 min but failed to show similar effect at 30 and 60 min. Similarly the compound **5d** showed moderate activity at 30 and 60 min but failed to show similar effect at 120 and 180 min whereas the compound **5e** with *N*-methyl piperazine substitution showed poor activity (table 2).

4. Conclusion

We have synthesized 1*H*-pyridine-4-yl-3,5-disubstituted indazoles and found **4b**, **4f** and **4g** exhibited potent anti-inflammatory activity and compounds **3a**, **4a**, **4b** and **5d** exhibited potent analgesic activity as that of standard drug indomethacin.

Supplementary Information

Copies of NMR spectra for compounds **3a–b**, **4a–g**, **5a–g** and **6a–b** are available at www.ias.ac.in/chemsci.

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References

1. (a) Zhao Q, Du J, Gu H, Teng X, Zhang Q, Qin H and Liu N 2007 *Pancreas* **34** 242; (b) Raffa D, Maggio B, Cascioferro S, Raimondi M V, Schillaci D, Gallo G, Daidone G, Plescia S, Meneghetti F, Bombieri G, Christiana A D, Pipitone R M, Grimaudo S and Tolomeo M 2009 *Eur. J. Med. Chem.* **44** 165
2. (a) Iwakubo M, Takami A, Okada Y, Kawata T, Tagami Y, Ohashi H, Sato M, Sugiyama T, Fukushima K and Iijima H 2007 *Bioorg. Med. Chem.* **15** 350; (b) Meyers M J, Arhancet G B, Heckerman S L, Chen X, Long S A, Mahoney M W, Rico J R, Garland D J, Blinn J R, Collins J T, Yang S, Haung H, McGee K F, Wendling J M, Dietz J D, Payne M A, Homer B L, Heron M I, Reitz D B and Hu X 2010 *J. Med. Chem.* **53** 5979

3. (a) Rodgers J D, Johnson B L, Wang H, Erickson-Viitanen S, Klabe R M, Bacheler L, Cordova B C and Chang C H 1998 *Bioorg. Med. Chem. Lett.* **8** 715; (b) Shi J J, Ji F H, He P L, Yang Y X, Tang W, Zuo J P and Li Y C 2013 *Chem. Med. Chem.* **8** 722
4. Huang L J, Shih M L, Chen H S, Pan S L, Teng C M, Lee F Y and Kuo S C 2006 *Bioorg. Med. Chem.* **14** 528
5. (a) Veera Reddy A, Surendra Reddy G and Dubey P K 2014 *J. Heterocycl. Chem.* **51** 1311; (b) Veera Reddy A, Surendra Reddy G and Dubey P K 2013 *Synth. Commun.* **43** 2236; (c) Surendra Reddy G, Arunasree K M, Dubey P K and Veera Reddy A 2014 *J. Chem. Sci.* **4** 1055
6. Veera Reddy A, Surendra Reddy G and Dubey P K 2011 *J. Heterocycl. Chem.* **48** 961
7. (a) Jones G 1996 In *Comprehensive Heterocyclic Chemistry II* A R Katritzky, C W Rees and E F V Scriven (Eds.) (Oxford: Pergamon) p. 167; (b) Balasubrahmanian M and Keay J G 1996 In *Comprehensive Heterocyclic Chemistry IIA* R Katritzky, C W Rees and E F V Scriven (Eds.) (Oxford: Pergamon) p. 245; (c) Kumar R and Chandra R 2001 *Adv. Heterocycl. Chem.* **78** 269; (d) Gavin D H 2004 *Tetrahedron.* **60** 6043
8. Woods K W, Fischer J P, Claiborne A, Li T, Thomas S A, Zhu G D, Diebold R B, Liu X, Shi Y, Klinghofer V, Han E K, Guan R, Magnone S R, Johnson E F, Bouska J J, Olson A M, Jong R D, Oltersdorf T, Luo Y, Rosenberg S H, Girandaa V L and Li Q 2006 *Bioorg. Med. Chem.* **14** 6832
9. D'Amour F E and Smith D L 1941 *J. Pharmacol. Exp. Ther.* **72** 74
10. Winter C A, Risley E A and Nuss G W 1962 *Proc. Soc. Exp. Biol. Med.* **111** 544
11. Antilla J C, Baskin J M, Barder T E and Buchwald S L 2004 *J. Org. Chem.* **69** 5578
12. Anuradha G, Vasuki G, Surendra Reddy G, Veera Reddy A and Dubey P K 2014 *Cryst. Rep.* **4** 527