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Novel synthesis of known carcinogen precursors

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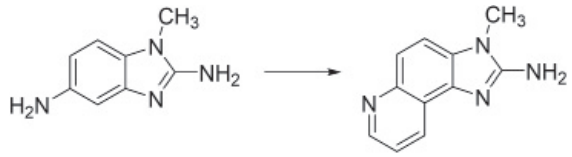
Abstract: 5-Amino-1-methylbenzimidazole (**2**) prepared from 2,4-dinitrochlorobenzene in four steps is a suitable reagent for nucleophilic vinylic substitution with various enolethers (**3**) thus affording *N*-substituted enaminoesters — aminoethylene derivatives (**4**). Their thermal cyclocondensation reaction under the Gould-Jacobs protocol gives regioselectively angularly annelated 8-substituted imidazo[4,5-*f*]quinolones (**5**), whose mechanism of origin has been attempted. The obtained results give suitable structures for the synthesis of food-borne carcinogen IQ (**1**).

Keywords: enaminoesters, food-borne carcinogens, Gould-Jacobs reaction, imidazoquinoline, IQ, ^{1}H and ^{13}C NMR

Introduction

Food-borne carcinogen 3-methyl-3*H*-imidazo[4,5-*f*] quinolin-2-amine (IQ, **1**) isolated from thermally treated meat has been prepared by derivatisation or transformation of the imidazole ring (Milata, 2001; Lakshmi, 2004). Our research group simplified this method (Bella, 2012) by developing a convenient and direct IQ synthesis, which involves the incorporation of a pyridine ring in the last reaction step. This synthesis starts from 2,5-diamino-1-methylbenzimidazole (Fig. 1):



1, IQ

Fig. 1. Preparation of food-borne carcinogen (**1**).

If amination of the imidazole ring in position 2 is considered as the final transformation, then various 3-methylimidazo[4,5-*f*]quinolines (**5**) should be suitable precursors for the synthesis of carcinogen IQ (**1**). This synthesis involves the transformation of fused quinolone ring to quinoline (Milata, 1988) in the penultimate reaction step.

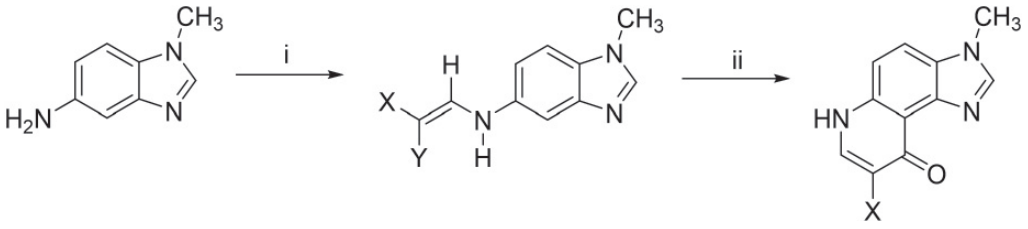
Material and Methods

Methods

All NMR spectra were obtained using an INOVA NMR 300 MHz spectrometer (operating frequencies: 300 MHz (^{1}H) and 75 MHz (^{13}C), equipped with an inverse triple resonance probe and a standard tuneable X/H probe. Tetramethylsilane was used to calculate the ^{1}H and ^{13}C chemical shift scales and for correct reference using the (residual) solvent signals (2.50 and 39.52 ppm for DMSO-d_6, and 7.26 and 77.00 ppm for $CDCl_3$).

Chemicals

Solvents and Dowtherm® were distilled before use. Enolethers (**3**) (Fig. 2) were commercially accessible



2 **4** **5**

Fig. 2. Reaction scheme of the preparation of compounds **4** and **5**.
i: RO—CH═CXY; **3**, **4**, **5a**: X—Y = $(COO)_2CMe_2$ (in this case for **5**: X = H), b: X = Y = COOMe, c: X = Y = COOEt, d: X = COMe, Y = COOMe, e: X = CN, Y = COOEt, ii: 250 °C, Dowtherm.

(**3a**, **3d**) or prepared according to known procedures (Milata, 1989; 2005) using tri(m)ethyl orthoformate and corresponding active methylene compounds (methyl acetoacetate (**3b**), ethyl cyanoacetate (**3c**) and Meldrum's acid (**3e**), respectively). Stannous chloride, hydrochloric acid, and sodium hydroxide were obtained from local vendors.

*5-Amino-1-methylbenzimidazole (**2**)*

Stannous dichloride dihydrate (21 g, 0,093 mol) dissolved in concentrated hydrochloric acid (36 %, 90 mL) was added in parts to stirred 5-nitro-1-methylbenzimidazole (5 g, 0,028 mol). The reaction mixture was refluxed for 5 h at approx. 150 °C in an oil bath. Subsequently, the reaction mixture was allowed to cool to laboratory temperature and precipitated 5-amino-1-methylbenzimidazole hydrochloride was filtered off, washed with ethanol, and dried with the yield of about 4,7 g (90 %). This hydrochloride was neutralised with sodium hydroxide solution (10 %, 1 g/5 ml) and the obtained solution was evaporated to dryness on a rotary vacuum evaporator (RVO) while solid residue was extracted using dichloromethane (4 × 30 ml). Collected extracts were evaporated to dryness on RVO. Yield 3,7 g (89 %), m.p. 165 °C (ethanol) (156—157 °C Ellis, 1974 (benzene)).

^{1}H NMR (DMSO — d_6): 3.71 (3H, s, CH_3); 4.74 (2H, s, NH_2); 6.61 (1H, dd, $^3J_{6,7}$ = 8.5 Hz, $^4J_{6,4}$ = 2.0 Hz, H-6); 6.77 (1H, d, $^3J_{4,6}$ = 2.0 Hz, H-4); 7.19 (1H, d, $^3J_{7,6}$ = 8.5 Hz, H-7); 7.89 (1H, s, H-2)

*General procedure to prepare 5-aminoethylene derivatives (**4**)*

To a suspension of 2 (1 g, 0,006 mol) in methanol or ethanol (60 mL), according to the alkoxygroup in **3,** enolether (**3**) was added (0,007 mol: **3a** — 1,40 g; **3b** — 1,38 g; **3c** — 1,04 g; **3d** — 1,15 g; **3e** — 1,54 g). The reaction mixture was refluxed for 0,5 — 2 hours (**3a** — 0,5 h, **3b** — 0,5 h, **3c** — 2 h, **3d** — 0,5 h, **3e —** 2 h) and monitored using TLC (eluent chloroform : methanol 9 : 1). After cooling of the reaction mixture, the separated crystals were filtered off and washed with cold methanol or ethanol, respectively, and dried. Yields and melting points are presented in Tab. 1:

*General procedure to synthesize 8-substituted imidazo[4,5-f]quinolines (**5**)*

A mixture of derivative 4 and Dowtherm® (15—50 ml) was refluxed at 250—260 °C for 1—9 hours (see Table 2). Reaction progress was monitored by TLC (chloroform : methanol 5 : 1). After cooling of the reaction mixture, the precipitated solid was filtered off, washed by hexane, and dried in vacuum at 80 °C for 6 h to remove traces of Dowtherm. The yields and melting points of the products (**5**) are provided in Tab. 2.

Results and discussion

The starting 5-amino-1-methylbenzimidazole (**2**) has been prepared according to a known procedure (Ellis, 1974) using cheap and commercially available 2,4-dinitrochlorobenzene treated with aqueous methylamine, the nitro group in position 2 was partially reduced with sodium hydrogen sulphide (Zinnin reduction) and the obtained 2-amino-4-nitro-*N*-methylaniline was cyclised with formic acid in total yield of 75—85 %. Initially, 5-nitro-1-methylbenzimidazole was reduced catalytically with hydrogen using Raney-Ni as catalyst at slightly elevated pressure (about 20 kPa). However, the results were not satisfactory, most likely due to

Tab. 1. Yields and melting points of 5-aminoethylene derivatives (**4**).

Compound	3a	3b	3c*	3d	3e**
Yield (%)	70	93	66	82	60
M.p. (°C)	170—172	224—227	180—183	209—212	127—130

See also: *Milata, 1989 ; **Ilavský, 1985.

Tab. 2. 8-Substituted imidazo[4,5-*f*]quinolines (**5**).

Starting derivatives 4	Dowtherm (ml)	Reflux (h)	Yield (%)	M.p. (°C)
4a	15	2	60	245—250
4b	15	0,5	60	255—257
4c	30	3	47	248—252*
4d	50	8,5	40	> 360
4e	20	1,5	50	245—248**

See also: *Milata, 1994; **Ilavský, 1985.

the presence of traces of sulphur or some sulphur-containing compounds poisoning the catalyst. Therefore, stannous dichloride in hydrochloric acid was used to obtain the starting raw material (**2**) more efficiently and in greater amounts.

A set of 8-substituted-3-methylimidazo[4,5-*f*]quinolines (**5**) was prepared, with 5-aminoethylene derivatives of 1-methylbenzimidazole (**4**) as appropriate building blocks for this synthesis. Derivatives **4** were obtained by the reaction of amine **2** and enolethers **3** (Fig. 2).

Due to the possible transesterification, enolethers with the same alkoxygroup attached to a tertiary carbon atom as the alkoxygroups on the ester moiety (**3a—d**) were used. Even though this group is replaced in a nucleophilic vinylic substitution in the first step of the reaction to avoid any possible transesterification, alcohol with the same alkoxygroup was used as a reaction solvent. Nucleophilic vinylic substitution (S_NV) of unequally substituted enol ethers (**3b**, **3c**) takes place with retention of the configuration (Saloň, 2005). These aminoethylenes (**4b**, **4c**) display geometrical isomerism of the double bond (Couchouron, 1983; Milata, 1989; Saloň, 2005) and antiperiplanar configuration of the aminomethylene fragment —NH—CH═ with $^3J_{HH}$ between 10.85—12.84 Hz stabilised with an intramolecular hydrogen bond between proton NH and carbonyl oxygen of the acetyl/alkoxycarbonyl group. In case of derivative **4b**, intramolecular bond with the acetyl group is preferred over the one with the methoxycarbonyl group (Couchouron, 1983; Milata, 1989) — Tabs. 3, 4 Figs. 3, 4:

Thermal cyclisation of **4** in inert media of Dowtherm under Gould-Jacobs reaction conditions afforded only angularly annelated imidazo[4,5-*f*]quinolines. This fact is confirmed by coupling constant $^3J_{HH}$ of the protons of the benzene ring (H_4, H_5 of about

Tab. 3: ^{1}H NMR spectra of compounds **4**.

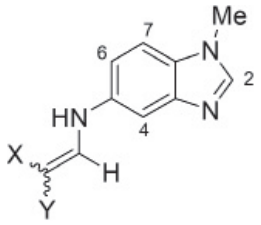


Fig. 3: Numbering of atoms of compounds **4**.

Compound	Solvent	E/Z (%)	H-2	H-4	H-6	H-7	—NH—	—CH═	X, Y	—CH_3
4a	DMSO	–	8.20 s	7.82 d ^4J = 2.1	7.44 dd $^3J_{6,7}$ = 8.7 $^4J_{6,4}$ = 2.1	7.61 d ^3J = 8.7	11.34 d ^3J = 14.5	8.58 d ^3J = 14.5	1.69 s	3.85 s
4b	$CDCl_3$	–	7.89 s	7.58 d ^4J = 2.0	7.13 dd $^3J_{6,7}$ = 8.6 $^4J_{6,4}$ = 2.0	7.37 d ^3J = 8.6	11.15 d ^3J = 13.8	8.61 s	3.87 s 3.85 s	3.78 s
4c*	DMSO	–	7.64 s	a	7.29 d $^3J_{6,7}$ = 8.6	7.57 d ^3J = 8.6	10.85 d ^3J = 14.0	8.44 d ^3J = 14.0	1.25t, 4.10q 1.26t, 4.20q ^3J = 7.02	3.82 s
4d	$CDCl_3$	E:(62) Z:(38)	7.92 s	7.56 d ^3J = 1.9	7.12 dd $^3J_{6,7}$ = 8.6 $^4J_{6,4}$ = 1.9	7.36 d ^3J = 8.6	12.84 d ^3J = 12.8 11.16 d ^3J = 12.8	8.51 s 8.66 d ^3J = 12.8	1.29t, 4.20q ^3J = 7.1 2.50 s 1.34t, 4.28q ^3J = 7.1 2.44 s	3.84 s
4e**	DMSO	Z:(53) E:(47)	7.92 s	7.64 d ^3J = 2.0 7.79 d ^3J = 1.9	7.39 dd $^3J_{6,7}$ = 8.7 $^4J_{6,4}$ = 2.0 7.12 dd $^3J_{6,7}$ = 8.7 $^4J_{6,4}$ = 1.9	7.57 d ^3J = 8.7	a 11.16 d ^3J = 14.0	8.50 d ^3J = 9.7 8.63 d ^3J = 14.0	1.29t, 4.20q ^3J = 7.12 1.30t, 4.28q ^3J = 7.12	3.82 s

[a] signal not observed; See also: *Milata, 1994; **Ilavský, 1985.

Tab. 4. ^{13}C NMR spectra of compounds **4**.

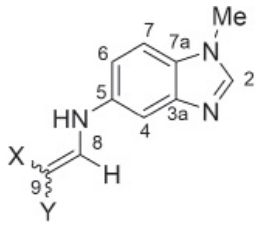


Fig. 4. Numbering of atoms of compounds **4**.

Compound	Solvent	C-2	C-3a	C-4	C-5	C-6	C-7	C-7a	C-8	C-9	Me	X, Y
4a	DMSO	143.8	141.8	104.0	138.9	120.1	114.6	133.1	153.3	98.9	30.8	26.5, 133.5, 162.7, 162.4
4b	$CDCl_3$	144.6	140.0	108.5	142.5	117.3	114.8	132.0	145.1	92.6	31.2	51.3, 166.1, 51.4, 169.5
4c*	DMSO	141.7	139.5	113.2	139.6	119.1	116.2	124.1	149.0	97.2	36.0	14.2, 62.7, 164.9, 64.4, 170.2
4d	$CDCl_3$	143.4	140.1	107.8	134.7	118.3	114.7	132.4	152.5	102.1	30.9	13.9, 31.5, 59.7, 166.6, 200.0
4e**	DMSO	143.7	141.1	107.9	141.7	118.6	115.3	132.5	153.3	73.5	29.9	14.3, 59.4, 115.3, 164.0

See also: *Milata, 1994; **Ilavský, 1985.

9 Hz, Tab. 5, Fig. 6). Similar NMR attributes were found in many previous experiments (Milata, 1987; 1989; 1994; 2000; etc.). The direction of the ring closure occurs preferentially at the C_4-position of the benzimidazole ring which is explained by higher thermodynamically stabilised -complex of two resonance hybrids of the angularly annelated product against the less stabilised linear one (Ishiwata, 1969; Fig. 5):

High temperature of the cyclisation reaction is necessary to eliminate alcohol from the alkoxycarbonyl group of the enaminoester fragment thus producing iminoketene which attacks electron-rich carbon C_4 of the enamine system —N—C_5=C_4. Hydrogen for alcohol production comes from the —NH— group and therefore cyclisation in inert media is possible only if the nitrogen atom is unsubstituted (Ishiwata, 1969; Milata, 2000). For iminoketene formation, reaction temperature close to 250 °C and dilution are very important. Very reactive cyanoiminoketene **4c** (X = CN) requires dilution of 1 g of the substrate/50 ml of Dowtherm, less reactive acetyl analog **4b** (X = $COCH_3$): 1 g/30 ml and ester and their equivalent (**4a**, **4d**, **4e**) 1 g/15–20 ml. Higher dilution complicates product isolation. Upscaling of the reaction requires lower dilutions.

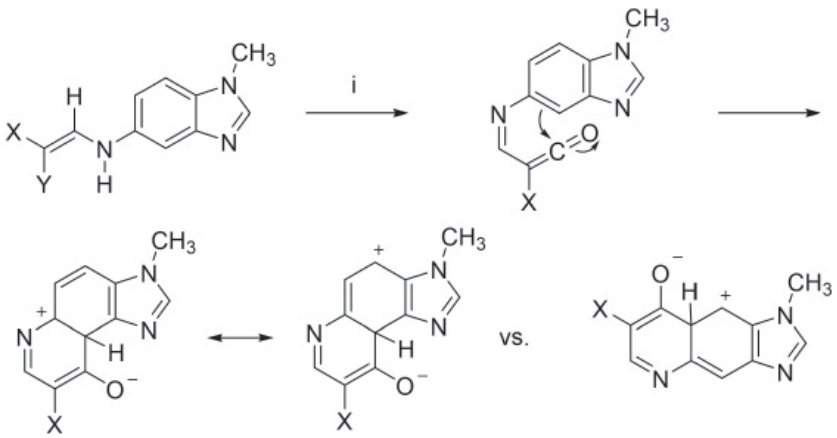


Fig. 5. Proposed mechanism explaining regioselectivity of the cyclisation reaction of compounds **4**. i: –250 °C: —ROH.

Tab. 5: ^{1}H NMR spectra of compounds **5**.

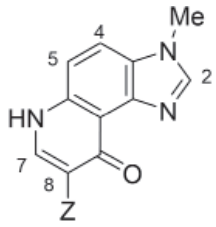


Fig. 6: Numbering of atoms of compounds **5**.

Compound	Solvent	H-2	H-4	H-5	H-7	H-8	Me	—NH—	Z
5a	DMSO	9.04 s	8.06 d ^{3}J = 9.4	8.57 d ^{3}J = 6.7	8.17 d ^{3}J = 9.4	7.37 d ^{3}J = 6.7	4.05 s	11.34 s	–
5b	TFA	9.34 s	8.33 d ^{3}J = 9.5	8.47 d ^{3}J = 9.5	9.32 s	–	4.04 s	11.34 s	4.23 s
5c*	TFA	9.25 s	8.22 d ^{3}J = 9.6	8.35 d ^{3}J = 9.6	9.23 s	–	4.13 s	11.34 s	1.24t, 4.42q ^{3}J = 7.3
5d	TFA	9.29 s	8.15 d ^{3}J = 9.3	8.28 d ^{3}J = 9.3	9.14 s	–	4.04 s	11.30 s	2.56 s
5e**	DMSO	8.54 s	7.52 d ^{3}J = 8.8	7.98 d ^{3}J = 8.8	8.32 s	–	3.93 s	10.70 s	–

See also: *Milata, 1994; **Ilavský, 1985.

Tab. 6: ^{13}C NMR spectra of compounds **5**.

Compound	Solvent	C-2	C-3a	C-4	C-5	C-5a	C-7	C-8	C-9	C-9a	C-9b	Me	Z
5a	DMSO	143.7	123.2	122.5	118.9	141.5	133.1	110.9	171.4	111.4	127.3	36.1	–
5d	DMSO	144.6	125.1	122.1	118.2	141.5	133.4	112.1	174.5	110.8	127.4	35.7	27.0, 205.0, 206.1

Compounds **5b**, **5c**, **5e** are insoluble in DMSO, $CDCl_3$ or TFA.

Conclusions

The Gould-Jacobs reaction has been proven to be an appropriate pathway to fuse a 3-substituted 4-pyridine nucleus to the starting amine, in our case 5-amino-1-methylbenzidazole (**2**). Therefore, 5-amino-1-methylbenzimidazole with a suitable type of enolether can be used to prepare intermediates for food-borne carginogen IQ (**1**). These intermediates were subsequently subjected to thermal cyclocondensation in inert media. A cyclisation mechanism similar to other types of pyridine nucleus fusion (like Skraup, Combes, Conrad-Limpach, Knorr, Meth-Cohn, Doebner reactions, etc. Li (2005)) has been proposed. At the same time, regioselectivity of the thermal cyclocondensation reaction has been explained and all conditions important for successful nuclei transformations studied have been described.

In particular, the potential of nucleus transformation into unsubstituted parent heterocycle was applied to our previous methodology (Milata, 1988) and subsequent amination of the 1-methylimidazole ring showed new perspective for the preparation of food-borne carcinogen IQ (**1**) (Waterhouse, 1985), thus opening a new synthetic route, an alternative to our synthesis (Bella, 2012) or classical methods based on cyclisation of 5,6-diaminopyridines (Milata, 2001). The prepared precursors and IQ (**1**) may have application in characterising chemical carcinogens in cancer hazard identification studies or in describing mechanisms of chemical carcinogenesis (Guyton, 2018).

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