Analgesic and Anticonvulsant Activities of Some Newly Synthesized Trisubstituted Pyridine Derivatives

Osama I. Abdel Salam[a], Mohamed A. Al-Omar[b], Nagy M. Khalifa[b], Abd El-Galil E. Amr[a,b,*], and Mohamed M. Abdallah[c]

[a] Applied Organic Chemistry Department, National Research Center, Dokki, Cairo, Egypt
[b] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia. Fax: +966-1-4676220. E-mail: aeamr1963@yahoo.com
[c] Research Unit, Saco Pharm. Co., 6th October City, Egypt

* Author for correspondence and reprint requests

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A series of novel pyridine carbohydrazide derivatives were synthesized from the reaction of 2-chloro-6-hydrazino-isonicotinic acid hydrazide with selected active reagents. All prepared compounds were tested as analgesic and anticonvulsant agents. The pharmacological screening showed that many of these compounds have good activities comparable to those of valdecoxib and carbamazepine as reference drugs.

Key words: Citrazinic Acid, Hydrazide, Hydrazones, Analgesic and Anticonvulsant Agents

Introduction

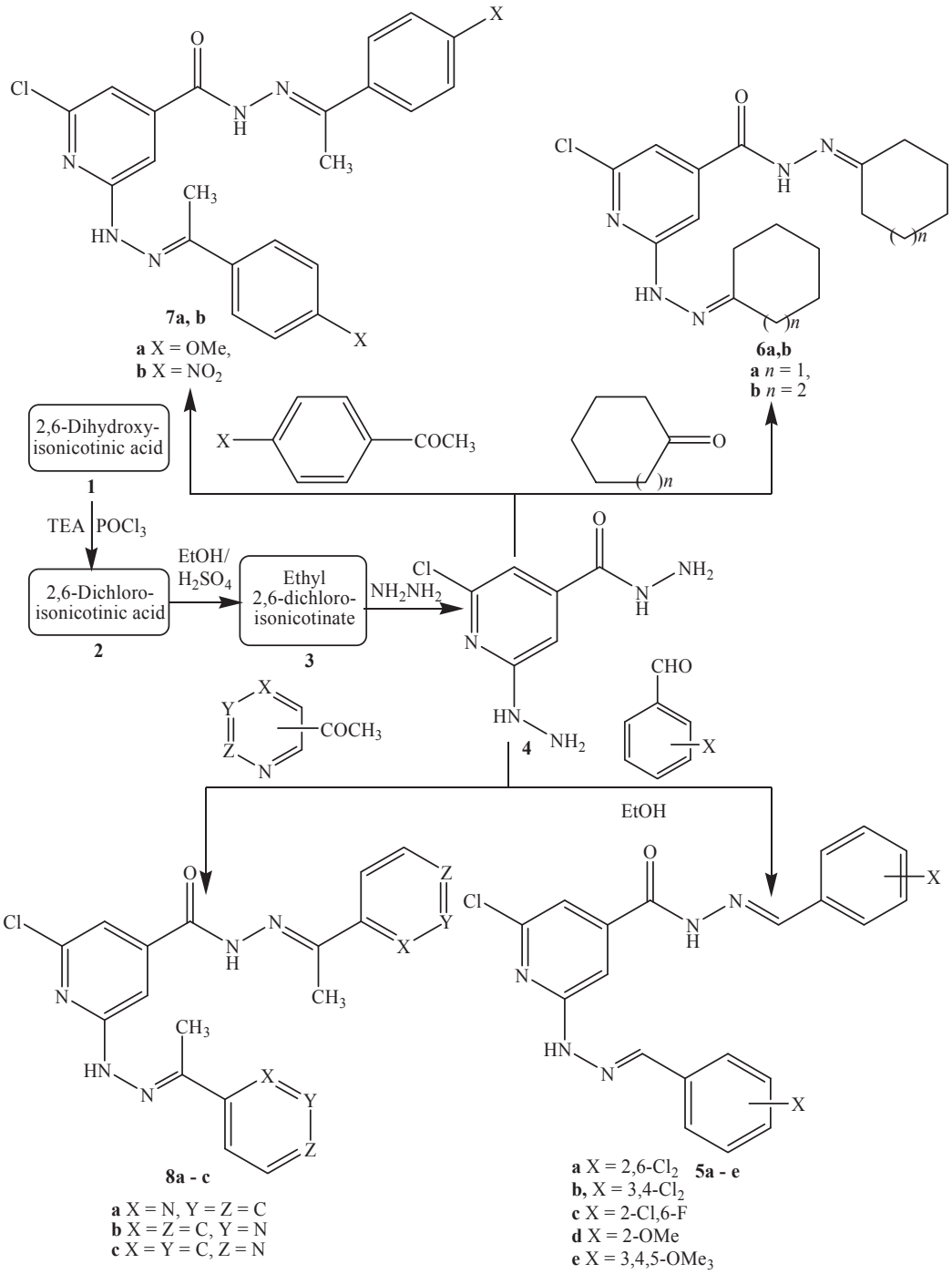
Several publications reported isonicotinic acid hydrazide and its derivatives as antitubercular (Tostmann *et al*., 2008; Anderson *et al*., 1956; Sarich *et al*., 1995), virucide, and bactericide (Vishnu *et al*., 1987) agents. In our previous work, we reported the synthesis, characterization, and a preliminary biological activity screening of some series of substituted pyridine derivatives as antimicrobial agents (Amr *et al*., 1999; Attia *et al*., 2000). Also, we found that certain substituted pyridines and their amide derivatives exhibit analgesic and anticonvulsant (Al-Omar *et al*., 2010), antimicrobial (Amr *et al*., 2003), and antitumour activities (Amr *et al*., 2006a, b). In addition, the biological and analgesic activities of many heterocyclic compounds containing a sulfur atom have been reviewed (Lorrain *et al*., 2003). On the other hand, some of the nitrogenous candidates have promising biological (Coetzee *et al.*, 2011) and anticancer activities (Amr *et al*., 2006a, b). Recently, some new Schiff base derivatives have been synthesized (Al-Omar and Amr, 2010; Al-Salahi *et al*., 2010) and tested as antimicrobial agents. In continuation of our previous work aiming at the synthesis of heterocyclic systems with remarkable biological activities, we report here the synthesis of some new pyridine derivatives with different substitution patterns at positions 2, 4, and 6. The present report also involves the analgesic and anticonvulsant activities study in comparison to valdecoxib® and carbamazepine® as standard drugs.

Results and Discussion

Chemistry

A series of derivatives **5**–**8** (Scheme 1) were synthesized in advance and screened as antimicrobial agents (Abdel Salam *et al*., 2013). Herein, we used these compounds for evaluation as analgesic and anticonvulsant agents.

2-Chloro-6-hydrazino-isonicotinic acid hydrazide (**4**) was synthesized according to the reported procedure (Tgolsen *et al*., 1991). Chlorination of 2,6-dihydroxy-isonicotinic acid (**1**) with phosphorus oxychloride afforded the corresponding 2,6-dichloro-isonicotinic acid (**2**), which was esterified with absolute ethanol in the presence of concentrated sulfuric acid to afford ethyl 2,6-dichloro-isonicotinate (**3**). The ester **3** was treated with hydrazine hydrate in refluxing ethanol to afford 2-chloro-6-hydrazino-isonicotinic acid hydrazide (**4**) in pure form and good yield. The hydrazone derivatives **5a**–**e** were synthesized *via* simple condensation of the hydrazide **4** with appropriate substituted aromatic aldehydes, namely 2,6-dichlorobenzaldehyde, 3,4-dichlorobenzaldehyde, 2-chloro-6-flourobenzaldehyde, 2-methoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde in refluxing absolute ethanol. Condensation of the same hydrazide **4** with selected



Scheme 1. Synthetic route for the synthesis of the target compounds **5**–**8**.

ketones, namely cycloalkanones (cyclohexanone or cycloheptanone), substituted acetophenone or acetyl pyridine in refluxing ethanol in the presence of a few drops of glacial acetic acid afforded the corresponding condensed derivatives **6a**, **b**, **7a**, **b**, and **8a–c**, respectively (Scheme 1).

Pharmacological screening

The tested two pharmacological properties, *i.e.* analgesic and anticonvulsant, have a neurological basis despite of their different biological receptors. Ten representative compounds (**5a**, **5b**, **5c**, **5d**, **5e**, **6b**, **7b**, **8a**, **8b**, and **8c**) were studied with respect to these properties.

Analgesic activity

All compounds tested exhibited analgesic activities in a hot-plate assay (Table I). The most potent were compounds **5c** and **8a** showing higher activities than valdecoxib, by nearly 130–160% (**5c** showed the most pronounced effect). Also, the analgesic activities of **5a**, **5b**, **5d**, **5e**, **6b**, **7b**, **8b**, and **8c** approached those of valdecoxib, and showed 60–95% activity as compared to this standard drug (100% activity) (Table I).

Anticonvulsant activity

Antagonism against yohimbine-induced clonic seizures in mice is considered a predictive model of anticonvulsant and *GABA*-mimetic potential (Dunm and Fielding, 1987). Compounds **7b** and **6b** were devoid of anticonvulsant activity in the yohimbine-induced clonic seizures assay, while compounds **5b**, **5d**, and **5e** showed interesting anticonvulsant activities. Their relative potencies compared to carbamazepine (1.0) were 0.78, 0.85, and 0.73, respectively. Compounds **5a**, **5c**, **8a**, **8b**, and **8c** were more potent than carbamazepine with relative potencies of 1.83, 2.05, 2.20, 2.24, and 1.78, respectively (Table II). ED_{50} values were determined as those doses which protected 50% of the tested animals against the convulsions induced by yohimbine.

Conclusion

The purpose of the present study was to examine whether structural modification of substituted pyridines might result in the detection of new potential analgesic and anticonvulsant reagents. A series of compounds were prepared and assayed in a variety of biological tests for analgesic and anticonvulsant the activities. The data

Table II. Anticonvulsant activities of selected compounds (as ED_{50} values), antagonizing yohimbine-induced clonic seizure, relative to the anticonvulsant activity of carbamazepine.

Compound	ED_{50} ± SE (mg/kg BW)	Relative potency compared to carbamazepine ± SE
Control	0	0
Carbamazepine	29 ± 0.31	1.0 ± 0.01
5a	15 ± 0.120	1.83 ± 0.0178
5b	36 ± 0.35	0.78 ± 0.007
5c	15 ± 0.114	2.05 ± 0.022
5 d	32 ± 0.30	0.85 ± 0.012
5e	56 ± 0.45	0.73 ± 0.010
6b	No protection	No protection
7b	No protection	No protection
8a	14 ± 0.118	2.20 ± 0.024
8b	12 ± 0.110	2.24 ± 0.020
8c	14 ± 0.118	1.78 ± 0.0180

Table I. Analgesic activities of the newly synthesized compounds **5a–e**, **6b**, **7b**, and **8a–c**.

Compound	Analgesic potency relative to valdecoxib ± SE						
	10 min	20 min	30 min	45 min	60 min	90 min	120 min
5a	0.61 ± 0.011	0.65 ± 0.011	0.74 ± 0.012	0.75 ± 0.018	0.77 ± 0.011	0.77 ± 0.011	0.77 ± 0.013
5b	0.61 ± 0.012	0.73 ± 0.012	0.79 ± 0.001	0.81 ± 0.015	0.84 ± 0.016	0.84 ± 0.016	0.84 ± 0.035
5c	1.27 ± 0.180	1.42 ± 0.160	1.43 ± 0.130	1.44 ± 0.190	1.42 ± 0.318	1.44 ± 0.288	1.40 ± 0.270
5d	0.77 ± 0.012	0.85 ± 0.014	0.84 ± 0.012	0.87 ± 0.015	0.88 ± 0.018	0.84 ± 0.012	0.83 ± 0.018
5e	0.88 ± 0.011	0.89 ± 0.011	0.89 ± 0.011	0.91 ± 0.017	0.92 ± 0.016	0.93 ± 0.015	0.91 ± 0.016
6b	0.66 ± 0.012	0.63 ± 0.012	0.88 ± 0.012	0.88 ± 0.016	0.88 ± 0.021	0.89 ± 0.017	0.89 ± 0.018
7b	0.91 ± 0.011	0.92 ± 0.009	0.93 ± 0.016	0.88 ± 0.019	0.83 ± 0.021	0.79 ± 0.016	0.65 ± 0.012
8a	0.97 ± 0.012	0.98 ± 0.015	1.40 ± 0.13	1.54 ± 0.210	1.57 ± 0.350	1.59 ± 0.340	1.40 ± 0.450
8b	0.63 ± 0.009	0.64 ± 0.017	0.73 ± 0.013	0.73 ± 0.018	0.74 ± 0.019	0.75 ± 0.016	0.78 ± 0.013
8c	0.82 ± 0.014	0.90 ± 0.016	0.92 ± 0.017	0.95 ± 0.021	0.96 ± 0.032	0.94 ± 0.018	0.93 ± 0.026
Valdecoxib	1.0	1.0	1.0	1.0	1.0	1.0	1.0

reported in Tables I and II shows that effect of variation in the chemical structure on the activity was rather unpredictable. Seldom did a particular structural modification lead to uniform alteration in the activity in all tests. However, some point of interest did emerge and a few generalizations can be made. The results of this investigation revealed that the observed increase in analgesic and anticonvulsant activities can be attributed to the presence of a chlorine atom in the pyridine ring at position 2 of the synthesized compounds. Obviously, the comparative evaluation of active compounds will require further studies; the data reported in this article may be a helpful guide for the medicinal chemist working in this area.

Experimental

Instrumentation

Melting points were not corrected and determined in open glass capillaries using an Electrothermal IA 9000 Series digital melting point apparatus (Stone, Essex, UK). Elemental analyses were performed with all final compounds using a Vario EL microanalytical unit (Elementar Analysensysteme, Hanau, Germany) at Cairo University, Cairo, Egypt, and were in good agreement (± 0.4%) with the calculated values. The IR spectra (in KBr) were recorded on an FT IR-8201 PC spectrophotometer (Shimadzu, Tokyo, Japan). The NMR spectra were measured with a Jeol 270-MHz spectrometer (FTGNM-EX 270; Tokyo, Japan) in DMSO-d_6 as solvent. The chemical shifts were recorded relative to tetramethylsilane (TMS). The mass spectra (EI) were run at 70 eV with a Finnigan SSQ 7000 spectrometer (Thermoinstrument System Inc., New Orleans, LA, USA), *m/z* values were indicated in Dalton. Thin-layer chromatography (TLC) (silica gel, aluminum sheets 60 F_{254}; Merck, Darmstadt, Germany) was used for tracing the reactions. Synthesis, physicochemical and spectral data of the compounds have been reported in advance (Abdel Salam *et al.*, 2013).

Chemistry

2-Chloro-6-hydrazino-isonicotinic acid hydrazide (**4**) was synthesized according to the reported procedure (Tgolsen *et al.*, 1991). The hydrazide derivatives **5a–e** were synthesized via simple condensation of the hydrazide **4** with appropriate substituted aromatic aldehydes, namely 2,6-dichlorobenzaldehyde, 3,4-dichlorobenzaldehyde, 2-chloro-6-flourobenzaldehyde, 2-methoxybenzaldehyde or 3,4,5-trimethoxybenzaldehyde in refluxing absolute ethanol. Condensation of the same hydrazide **4** with selected ketones, namely cycloalkanones (cyclohexanone or cycloheptanone), substituted acetophenone or acetyl pyridine in refluxing ethanol in the presence of a few drops of glacial acetic acid afforded the corresponding condensed derivatives **6a**, **b**, **7a**, **b**, and **8a–c**, respectively (Scheme 1).

Pharmacological screening

Animals

Biological experiments were conducted according to the ethical rules, and animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. Approval of the institutional animal ethical committee for animals studies was obtained from the Office of Environmental Health and Radiation Safety, ACUC Protocol 1096–5. All animals were allowed free access to water and were kept on a constant standard diet.

Analgesic activity

Sixty Webster mice of both sexes, weighing 20–25 g, were divided into 10 groups. One group was kept as control (receiving saline), the second group received vehicle (gum acacia), and the third one received valdecoxib as a reference drug, whereas the other groups received the test compounds by subcutaneous administration [dose 5 mg/kg body weight (BW)]. Mice were dropped gently in a dry glass beaker of 1 L capacity maintained at 55–55.5 °C. Normal reaction time in seconds for all animals was determined at time intervals of 10, 20, 30, 45, 60, 90, and 120 min. This is the interval extending from the instant the mice reach the bottom of the hot beaker till the animals lick their feet or jump out of the beaker (Austen and Brocklehurst, 1961). Potencies relative to that of valdecoxib were determined (Table I).

Anticonvulsant activity

Male Webster mice, weighing 20–30 g, were individually placed in a clear plastic cylinder, and the test compounds were administered intraperitoneally (5 mg/kg BW), 30 min prior to a dose of 45 mg/kg BW of yohimbine-HCl. The animals

were observed for onset and number of clonic seizures (Baizer *et al*., 1956) (Table II). ED_{50} values for compounds with 95% confidence limits were calculated for the antagonism of yohimbine-induced clonic seizures according to Austen *et al.* (1961).

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