Full Paper

Synthesis and Biological Activity of Functionalized Indole-2-carboxylates, Triazino- and Pyridazino-indoles

Adel A. El-Gendy[1], Mohamed M. Said[2], Nagat Ghareb[2], Yasser M. Mostafa[3], and El Sayed H. El-Ashry[4]

[1] Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[2] Organic Chemistry Department, Faculty of Pharmacy, Suez-Canal University, Ismailia, Egypt
[3] Pharmacology Department, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt
[4] International Center for Chemical and Biological Sciences (HEJ Research Institute for Chemistry), Karachi University, Karachi, Pakistan

Condensation of aryl hydrazines with ethyl pyruvate gave the respective hydrazones **4**–**6**; Fischer indolization led to substituted-*1H*-indole-2-carboxylic acid ethyl esters **7**–**9**. The Mannich reaction of these compounds with formaldehyde and morpholine yielded ethyl 3-(morpholinomethyl)-substituted-*1H*-indole-2-carboxylates **10-12**. The 5,7-dichloro-1*H*-indole-2-carbohydrazide **13** was cyclized with methyl orthoformate in DMF to give 6,8-dichloro[1,2,4]triazino[4,5-*a*]indol-1(2*H*)-one **14**. Vilsmeier–Haack formylation of **7**–**9** gave ethyl 3-formyl-substituted-*1H*-indole-2-carboxylates **15**–**17** whose 2,2′-((5-chloro-2-(ethoxycarbonyl)-*1H*-indol-3-yl)methylene)bis-(sulfanediyl) diacetic acid **18** was prepared. The reaction of **15** and **16** with substituted anilines by conventional and microwave methods gave ethyl 3-(*N*-aryliminomethyl)-5-halo-*1H*-indole-2-carboxylates **19**–**29**. In a cyclocondensation reaction of **19**–**25** with thiolactic acid or thioglycolic acid substituted indolylthiazolidinones **30**–**33** were prepared. Reaction of hydrazine hydrate with **15**–**17** did not give the respective hydrazones but directly led to the cyclized products substituted-3*H*-pyridazino[4,5-*b*]indol-4(5*H*)-ones **34**–**36**, while a reaction with 2,4-dichlorophenylhydrazine yielded the uncyclized hydrazones. The chlorination of **35** and **36** with $POCl_3$ gave pyridazino[4,5-*b*]indoles **39** and **40**, respectively; reaction of the latter compounds with morpholine gave 4-(substituted-*5H*-pyridazino[4,5-*b*]indol-4-yl)morpholine **41** and **42**. Mannich reaction of **34** with formaldehyde and *N*-ethylpiperazine gave 8-chloro-3-((4-ethylpiperazin-1-yl)methyl)-3*H*-pyridazino[4,5-*b*]indol-4(5*H*)-one **43**. The microwave assistance of selected reactions has a profound effect on the reaction speed. The structures of the new compounds were confirmed by both analytical and spectral data. Some compounds were subjected to investigations concerning their antimicrobial, tranquilizing, and anticonvulsant activities.

Keywords: Fischer Indolization / Substituted phenylhydrazone / Schiff's base / Vilsmeier–Haack Formylation / Thiolactic acid

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Introduction

The incorporation of a nitrogen heterocycle on an aliphatic side chain at position-3 of the indole-2-carboxylate led to diverse biological effects as anti-inflammatory [1], selective D_4 ligands [2], and 5-HT_3 antagonists [3]. Indole compounds containing either linked or fused thiazolidinone moieties were found to possess antibacterial [4–5], fungicidal [6], antifertility [7], and anticonvulsant [8–9] activities. Likewise, the pyridazino[4,5-*b*]indole-ring sys-

Correspondence: E. S. H. El Ashry, Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
E-mail: Eelashry60@hotmail.com
Fax: +20 342 71360

tem has been known for several decades, and, so far, a variety of biological activities has been reported for its derivatives, such as antihypertensive [10 – 14], antiplatelet aggregation [15 – 17], and HIV-1 reverse transcriptase inhibitory [18] activities. In view of these findings, the present work includes the synthesis of some halogenated indole-2-carboxylates linked or fused to heterocycles such as morpholine, pyrazine, pyrazoline, 5*H*-pyridazino[4,5-*b*]indoles, and thiazolidin-4-ones and we tested them for their antimicrobial, tranquilizing, and anticonvulsant activities.

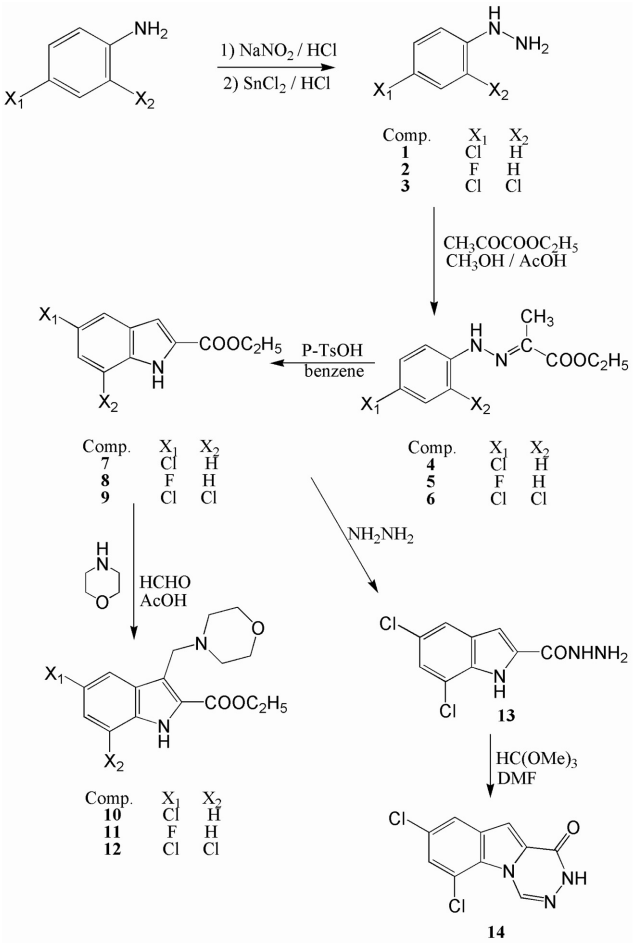
Results and discussion

Chemistry

In the present investigation, the synthesis of 4-chloro-, 4-fluoro-, and 2,4-dichloro-phenylhydrazines **1 – 3** was carried out by reduction of the diazonium salt of the respective substituted aniline with stannous chloride in acid medium [19]. Fischer indolization [20] of the corresponding ethyl pyruvate derivatives **4 – 6** [21] gave the substituted-*1H*-indole-2-carboxylic acid ethyl esters **7 – 9**. Reaction of 5,7-dichloro-*1H*-indole-2-carboxylic acid ethyl ester **9** with hydrazine hydrate gave 5,7-dichloro-*1H*-indole-2-carbohydrazide **13** [23], and refluxing with the one-carbon inserting reagent methyl orthoformate in DMF gave 6,8-dichloro-1,2,4-triazino[4,5-*a*]indole-1(2*H*)-one **14** (Scheme 1, [24]). The assignment of structure **14** for that product where the carbons were inserted between the hydrazine moeity and the indole NH rather than the indole carbon was based on the absence of the indole NH in its ^{1}H-NMR spectrum, and the presence of the proton on the indole C-10 (d 9.22 ppm).

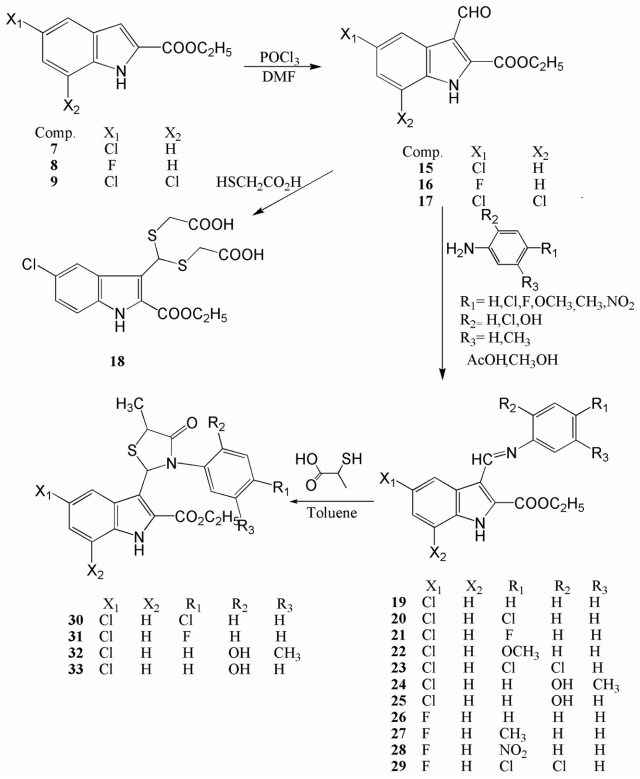
The Mannich reaction [22] of **7 – 9** with formaldehyde and morpholine in the presence of glacial acetic acid gave ethyl-substituted-3-(morpholinyl-methyl)-*1H*-indole-2-carboxylates **10 – 12**.

The Vilsmeier – Haack formylation [25 – 26] of **7 – 9** using $POCl_3$/DMF gave 3-formyl-substituted-*1H*-indole-2-carboxylic acid ethyl esters **15 – 17**. Refluxing a mixture of **15**, aniline and thioglycolic acid [7] gave 2,2′-((5-chloro-2-(ethoxycarbonyl)-*1H*-indole-3-yl)methylene)-bis(sulfanediyl)diacetic acid **18**, instead of the expected thiazolidinone which may be attributed to a faster reaction of the aldehyde with thioglycolic acid rather than with the amine. Thus, ethyl 3-(*N*-aryliminomethyl)5-halo-*1H*-indole-2-carboxylates **19 – 29** were prepared [27] by boiling the respective aldehyde with the substituted aniline in methanol in the presence of glacial acetic acid for six hours; microwave irradiation of a mixture of the two components for 5 min gave the same products. The indolylthiazolidinone derivatives, ethyl 5-chloro-3-(5-methyl-4-oxo-3-arylthiazolidin-2-yl)-*1H*-indole-2-carboxylates **30 – 33** were prepared by cyclocondensation of Schiff's bases **20**, **21**, **24** and **25** with thioglycolic acid [28 – 29] and thiolactic acid [30] (Scheme 2).



Scheme 1. Synthesis of indolyl heterocycles.

Substituted-3*H*-pyridazino[4,5-*b*]indol-4(5*H*)-ones **34 – 36** were prepared by boiling **15 – 17** with hydrazine hydrate for six hours with 80% yield; under microwave irradiation, only 3 min were required to reach better yields. On the other hand, reaction of 3-formyl-substituted-*1H*-indole-2-carboxylic acid ethyl esters **16** and **17** with 2,4-dichlorophenylhydrazine did not give the pyridazino derivatives, but the corresponding hydrazones **37** and **38**, respectivly, were formed. 4-Chloro-substituted-5*H*-pyridazino[4,5-*b*]indoles **39** and **40** were prepared by boiling **35** and **36** with $POCl_3$ for 10 h; their reaction with morpholine in DMF [31] led to the formation of 4-morpholino-substituted-5*H*-pyridazino[4,5-*b*]indoles **41** and **42**. Mannich condensation [32] of 5-chloro-3*H*-pyridazino[4,5-*b*]indol-4(5*H*)-one **34** with 4-ethylpiperazine and



Scheme 2. Synthesis and reactions of formyl indoles.

Table 1. Anticonvulsant activity.

Groups	Time of death after injection (min)	Significant [a] vs. Strychnine-injected group treated with vehicle	Significant [b] vs. phenobarbital	Significant [c] vs. diazepam
Vehicle	4.83 ± 0.0042	-	-	-
PB	9.61 ± 0.020	[a] ($p < 0.05$)	-	-
DZP	7.13 ± 0.036	[a] ($p < 0.05$)	-	-
30	5.83 ± 0.048	[a] ($p < 0.05$)	[b] ($p < 0.05$)	[c] ($p < 0.05$)
31	4.43 ± 0.028	[a] ($p < 0.05$)	[b] ($p < 0.05$)	[c] ($p < 0.05$)
32	3.63 ± 0.028	-	-	-
33	6.95 ± 0.035	[a] ($p < 0.05$)	[b] ($p < 0.05$)	[c] ($p < 0.05$)

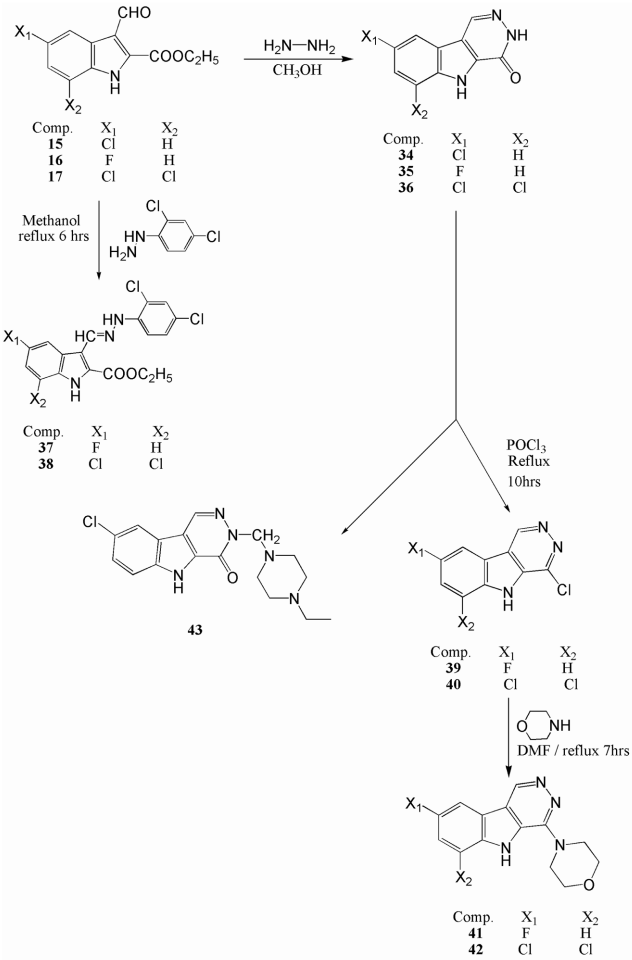
formaldehyde in ethanol gave 8-chloro-3-((4-ethylpiperazin-1-yl)methyl)-3*H*-pyridazino[4,5-*b*]indol-4(5*H*)-one **43** (Scheme 3).

Biological studies

Compounds **30**–**33** (10 mg/kg body weight) were evaluated for their anticonvulsant and tranquilizing activities following the methods of Kerley *et al*. [33] and Janssen *et al*. [34], respectively.

Anticonvulsant activity

The results showed that compounds **30**, **31**, and **33** have significant ($p < 0.05$) anticonvulsant activity. Convulsions



Scheme 3. Synthesis of pyridazino indoles.

were induced by strychnine (2 mg/kg body weight) injected intraperitoneally, the end point was calculated by occurence of death (time in minutes) after strychnine injection. On the other hand, compound **32** showed no anticonvulsant activity. Meanwhile, the anticonvulsant activities of compounds **30**, **31**, and **33** were less than the activity of phenobarbital sodium (PB) (200 mg/kg body weight) and diazepam (DZP) (1 mg/kg body weight) which were used as standards (Table 1).

Tranquilizing activity

The tranquilizing activity of the tested compounds **30**–**33** was studied by observing their effect on the behavior of mice while monitoring their activity in the catalepsy test [34]; chlorpromazine (CPZ) (4 mg/kg body weight) was used as reference standard. The number of animals that failed to remain on the rod for 3 min at intervals of 30, 60, 80, and 120 minutes after administration of the tested compounds were recorded and calculated as percentage. Failure of the mice to remain on the rod for

Table 2. Effect on motility using rotating rod.

Groups	Intervals (min)			
	30	60	90	120
Vehicle	188 ± 3.742	194 ± 5.99	212 ± 8.602	222 ±11.58

Table 3. Failure (%) before 180 seconds.

Compound	Failure (%)			
CPZ *	100%	100%	100%	100%
30	100%	100%	100%	100%
31	100%	100%	100%	100%
32	100%	100%	100%	100%
33	100%	100%	100%	100%

* CPZ, chlorpromazine

3 min indicated motor incoordination (Table 2). The results in Table 3 indicate that compounds **30**–**33** had incoordination behavior (tranquilizing activity).

Antimicrobial activity

The activities of compound **18**, Schiff's bases **19**–**29**, and the substituted indolylthiazolidinones **30**–**33** against representative *Gram*-positive and *Gram*-negative bacteria as well as fungi were tested by the disk-diffusion method [35]. The results showed that compound **18** had high activity against *Gram*-positive bacteria, while compounds **19**–**29** had moderate activity against *Gram*-negative bacteria and fungi; compounds **30**–**33** possess high activity against fungi and moderate activity against *Gram* positive strains (Table 4).

Conclusions

Indole 2-cartboxylates were used as precursors for the respective 3-formyl derivatives and indolyl heterocycles as well as pyridazino-indoles. A selected series was also synthesized under microwave irradiation which, as expected [40], saved much time. The physical data of the newly synthesized compounds is presented in Table 5. The anticonvulsant, tranquilizing, and antimicrobial activities of selected compounds showed promising results.

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Table 4. Antimicrobial activity of tested compounds*.

Compound	*Gram*-negative bacteria	*Gram*-positive bacteria		Fungi
	E. coli	*S. aureus*	*B. subtilis*	*C. albicans*
18	–	+++	+++	–
19	+	–	–	+
20	+	–	–	+
21	+	–	–	+
22	+	–	–	+
23	+	–	–	+
24	+	–	–	+
25	+	–	–	+
26	+	–	–	+
27	+	–	–	+
28	+	–	–	+
29	+	–	–	+
30	–	+	–	++
31	–	+	–	++
32	–	+	–	++
33	–	+	–	++

Solvent: DMF, [c] = 20 μL mL^{-1}. Rating: + = moderately active (inhibition zone 1 – 5 mm); ++ = more active (inhibition zone 5-10 mm); +++ = highly active (inhibition zone 10 – 15 mm); reference substance for bacteria: chloramphenicol; reference substance for fungi: nystatine.

Demonstrator of Microbiology, Faculty of Pharmacy, Suez Canal University, for carrying out the antimicrobial screening.

The authors have declared no conflict of interest.

Experimental

Chemistry

Melting points were measured in open capillary tubes using Stuart melting point apparatus SMP10 (Stuart Scientific. Stone, Staffordshire, UK) and are uncorrected. Infrared (IR) spectra were measured on a Vector 22 Infrared spectrophotometer (νmax in cm^{-1}; Bruker, Bioscience, Billerica, MA, USA). Proton magnetic resonance (^{1}H-NMR) spectra were recorded on Bruker AM 300 spectrometer (300 MHz). Chemical shifts are reported in δ values (parts per million, ppm) relative to tetramethylsilane (TMS) as internal standard and the coupling constant values are given in Hz. Abbreviation used in NMR analysis are as follows: d = doublet, dd = doublet of doublets, m = multiplet, q = quartet, s = singlet, t = triplet. Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT 312 mass spectrometer connected with a MASPEC Data System (Thermo Electron Corporation, Germany). High-resolution electron impact mass measurements (HREI-MS) were carried out on JEOL JMS HX 110 mass spectrometer (JEOL, Tokyo, Japan). Elemental analyses were performed in the Microanalytical center, Cairo University, Egypt. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel $60F_{254}$ (Merck, Germany) and visualized with UV light. Microwave irradiation was achieved using a domestic microwave oven MS-3443A (1000-W output power) frequency 2450 Hz, containing glass tray and rotating ring. Analytical data for the compounds is shown in Supplemental Material.

Table 5. Physical Data of Ethyl 3-(*N*-aryliminomethyl)-5-halo-indole-2-carboxylates **19–29**.

Compound	X_1	X_2	R_1^	R_2	R_3	Mp. (°C)	Yield (%)	
							Conventional	Microwave
19	Cl	H	H	H	H	195	70	*
20	Cl	H	Cl	H	H	192	75	*
21	Cl	H	F	H	H	187	75	*
22	Cl	H	OCH_3	H	H	230	80	*
23	Cl	H	Cl	Cl	H	260	82	*
24	Cl	H	H	OH	CH_3	170	60	*
25	Cl	H	H	OH	H	150	60	*
26	F	H	H	H	H	205	77	85
27	F	H	CH_3	H	H	216	75	88
28	F	H	NO_3	H	H	270	70	80
29	F	H	Cl	Cl	H	238	82	90

* This experiment was not carried out

Substituted phenylhydrazines ***1–3***

Concentrated HCl (100 mL) was added with stirring to a solution of the substituted aniline (40 mmol) in glacial acetic acid (20 mL) at room temperature. The reaction mixture was then cooled to 0°C and treated with sodium nitrite solution (2.76 g, 40 mmol in 8 mL water). The cold diazonium salt solution was rapidly filtered and treated dropwise with a cold solution of stannous chloride dihydrate (20 g) in conc. HCl (20 mL). The insoluble salt was collected by filtration and washed with saturated sodium chloride solution (30 mL). The substituted phenylhydrazine was liberated from the salt by treatment with aqueous sodium hydroxide (15%, 200 mL). The product was extracted twice with ether (200 mL). The combined extracts were washed with water and then dried over anhydrous Na_2SO_4. The ethereal solution was evaporated, then dried and recrystallized from ethanol. 4-Chlorophenyl hydrazine **1**: mp. 83°C, lit. [19] mp. 85–87°C (yield 60%). 4-Fluorophenyl hydrazine **2**: mp. 60°C, lit. [36] mp. 63–65°C (yield 50%). 2,4-Dichlorophenyl hydrazine **3**: mp. 93–95°C, lit. [37] mp. 94–95°C (yield 40%).

Ethyl-pyruvate-substituted phenylhydrazones ***4–6***

Ethyl pyruvate (1.3 g, 11 mmol) was added dropwise with stirring into an alcoholic solution of substituted phenylhydrazine **1–3** (11 mmol) in the presence of glacial acetic acid (0.5 mL). The resulting precipitate was further stirred for 15 minutes at room temperature and then left for complete precipitation. The product was filtered, dried, and recrystallized from ethanol. Ethyl pyruvate 4-chlorophenylhydrazone **4**, mp. 136°C, lit. [21] mp. 138°C (yield 74%).

Ethyl-substituted-1H-indole-2-carboxylates ***7–9***

A mixture of *p*-toluenesulfonic acid (3 g, 17.4 mmol) and dry benzene (50 mL) was heated under reflux using Dean–Stark apparatus for 1.5 h. A suspension of the hydrazone **4–6** (10 mmol) in dry benzene (30 mL) was added and the whole mixture was refluxed for 5 h. The resulting solution was diluted with benzene, washed with aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting product was crystallized from ethanol.

Ethyl 3-(morpholinyl-methyl)-substituted-1H-indole-2-carboxylates ***10–12***

A solution of the substituted-*1H*-indole-2-carboxylate **7–9** (0.26 mmol) in methanol (20 mL) was added to a well stirred mixture of formaline (0.02 mL), morpholine (0.52 mmol), and glacial acetic acid (3 mL). The mixture was then refluxed for 12 h. The resulting solution was evaporated to dryness. The product was crystallized from methanol.

5,7-Dichloro-1H-indole-2-carbohydrazide ***13***

A mixture of 5,7-dichloro-*1H*-indole-2-carboxylate **9** (61 mg, 0.0235 mmol) and hydrazine hydrate (2 mL, 80%) was mixed uniformly and irradiated by microwave for 3 min. After cooling, the product was crystallized from ethanol.

6,8-Dichloro-[1,2,4]triazino[4,5-a]indol-1(2H)-one ***14***

A mixture of 5,7-dichloro-*1H*-indole-2-carbohydrazide **13** (133 mg, 0.547 mmol), *N*,*N*-dimethylformamide (1 mL), and trimethyl orthoformate (0.089 mL, 0.84 mmol) was boiled for 6 h. The solvent was removed *in vacuo* and the residue was recystallized from a DMF/ethanol mixture.

Ethyl 3-formyl-substituted-1H-indole-2-carboxylates ***15–17***

In a 250 mL two-necked round-bottomed flask, dry *N*,*N*-dimethylformamide (2 mL, 0.026 mol) was cooled in an ice-bath for 30 min. Phosphorus oxychloride (0.7 mL, 0.004 mol) was dropped into the reaction flask in about 5min. The cooling bath was removed and the reaction mixture was then stirred at room temperature for 30 min. The mixture was newly cooled in an ice-bath and a solution of substituted-*1H*-indole-2-carboxylates **7–9** (4 mmol) in DMF (2 mL) was dropped into the reaction flask (taking about 5 min). The mixture was then stirred for 2 h at 80–100°C. The colored solution obtained was poured over crushed ice (20 g). The resulting orange precipitate was left overnight for complete precipitation. The collected product was filtered and washed first with warm H_2O and then with ethanol/water mixture. The resulting products were crystallized from ethanol.

2,2′-((5-Chloro-2-(ethoxycarbonyl)-1H-indol-3-yl)methylene)bis(sulfanediyl)diacetic acid ***18***

In a 250 mL two-necked round-bottomed flask, a mixture of ethyl 5-chloro-3-formyl-*1H*-indole-2-carboxylate **15** (100 mg, 0.4 mmol), thioglycollic acid (0.036 mL, 0.4 mmol), and dry toluene (30 mL) was heated under reflux for 12 h until the theoretical amount of water was collected azeotropically. The reaction mixture was cooled, washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting product was recrystallized from ethanol.

Ethyl 3-(N-aryliminomethyl)-5-halo-1H-indole-2-carboxylates ***19–29***

Conventional method

A mixture of ethyl 3-formyl-5-halo-*1H*-indole-2-carboxylate **15**–**16** (1 mmol) and glacial acetic acid (0.5 mL) was dissolved in methanol (10 mL) by heating. Then, the appropriate aniline derivative (1 mmol) was added to this solution and the whole solution was refluxed for 6 h. The hot solution was left at room temperature. The collected solid was filtered, dried, and recystallized from ethanol.

Microwave method

A mixture of ethyl 3-formyl-5-halo-*1H*-indole-2-carboxylate **15**–**16** (0.5 mmol), glacial acetic acid (1 drop), methanol (1 mL), and the appropriate aniline derivative (0.5 mmol) was mixed uniformly and irradiated by microwave for 5 min. After cooling, the product was crystallized from ethanol (Table 5).

Ethyl 3-(3-aryl-5-methyl-4-oxothiazolidin-2-yl)-5-chloro-1H-indole-2-carboxylates ***30–33***

In a 250 mL two-necked round-bottomed flask, a mixture of the appropriate ethyl 3-(*N*-aryliminomethyl)-5-chloro-*1H*-indole-2-carboxylate **20**–**21**, **24**–**25** (2 mmol), toluene (30 mL), and thiolactic acid (2 mmol) was heated under reflux using Dean–Stark apparatus for 32 h. The reaction mixture was cooled, washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting product was crystallized from ethanol.

Substituted-3H-pyridazino-[4,5-b]indol-4(5H)-ones ***34–36***

Conventional method

To a solution of ethyl 3-formyl-substituted-*1H*-indole-2-carboxylate **15**–**17** (0.18 mmol) in methanol (20 mL), 80% hydrazine hydrate (0.562 mmol) was added. The mixture was boiled for 6 h. The white product which formed was crystallized from DMF/ethanol mixture (yield 80%).

Microwave method

A mixture of ethyl 3-formyl-substituted-*1H*-indole-2-carboxylate **15**–**17** (0.09 mmol) and 80% hydrazine hydrate (0.281 mmol) was mixed uniformly and irradiated by microwave for 3 min. After cooling, the product was crystallized from DMF/ethanol (yield 93.44%).

Ethyl 3-((2-(2,4-dichlorophenyl)hydrazono)methyl)-substituted-1H-indole-2-carboxylates ***37–38***

Conventional method

2,4-Dichlorophenylhydrazine (0.09 mmol) was added to a solution of ethyl 3-formyl-substituted-*1H*-indole-2-carboxylate **16**, **17** (0.09 mmol) in methanol (10 mL) and the resulting reaction mixture was refluxed for 6 h. The solid that separated on cooling was recrystallized from ethanol (yield 80%).

Microwave method

A mixture of 3-formyl-substituted-*1H*-indole-2-carboxylic acid ethyl esters **16**–**17** (0.09 mmol), 2,4-dichlorophenylhydrazine (0.09 mmol), and methanol (2 mL) was mixed uniformly and irradiated by microwave for 5 min. After cooling, the product was crystallized from ethanol (yield 88%).

4-Chloro-substituted-5H-pyridazino[4,5-b]indoles ***39–40***

A mixture of substituted-3*H*-pyridazino[4,5-*b*]indol-4(5*H*)-ones **34**–**35** (1.1 mmol) and phosphorus oxychloride (20 mL) was refluxed at 300°C for 10 h. A dark-yellow-colored solution formed. It was cooled, then diluted with a large amount of diethyl ether (100 mL). The collected product was filtered and washed with cold H_2O and then dried. The resulting product was crystallized from a dichloromethane/methanol mixture.

4-(Substituted-5H-pyridazino[4,5-b]indol-4-yl)-morpholines ***41–42***

A mixture of 4-chloro-substituted-*5H*-pyridazino[4,5-*b*]indole **39**–**40** (0.09 mmol), morpholine (0.1 mmol) and dry *N*,*N*-dimethyl formamide (4 mL) was refluxed for 7 h. The reaction mixture was cooled, poured into crushed ice and finally left overnight. The collected solid was filtered, dried, and recrystallized from DMF / ethanol mixture.

8-Chloro-3-((4-ethylpiperazin-1-yl)methyl)-3H-pyridazino[4,5-b]indol-4(5H)-one ***43***

A well-stirred mixture of formaline (0.2 mL), *N*-ethyl piperazine (0.3 mmol), and glacial acetic acid (5 drops) was added to a suspension of 8-chloro-3*H*-pyridazino[4,5-*b*]indol-4(5*H*)-one **33** (0.26 mmol) in ethanol (20 mL). The whole mixture was then refluxed for 24 h. The resulting mixture was filtered, dried, and recrystallized from a DMF / ethanol mixture.

Biological studies

Compounds **30**–**33** were evaluated for their anticonvulsant, tranquilizing, and antimicrobial activities.The anticonvulsant activity was determined according to the method of Kerley *et al.* [33] using 28 male mice (20–25 g). The mice were divided into seven groups of four mice each. The first group served as control and was injected with the vehicle (1% Tween80). The second was given phenobarbital sodium (PB) (200 mg/kg b.wt.; ip). The third one was injected with diazepam (DZP) (1 mg/kg b.wt.; ip). The other groups were injected with the test compounds **30**–**33** (100 mg/kg b.wt.; ip). After 60 min., each mouse was injected with strychnine sulfate (2 mg/kg b.wt.; ip; LD99 for mice). The end point was to measure the ability of these four compounds to prolong life beyond the time of death observed after strychnine injection in comparison to the control animals. Compounds which provided significant anticonvulsant activity were addi-

tionally compared to the classical standard anticonvulsant drugs, PB and DZP.

The activities of compound **18**, Schiff's bases **19** – **29**, and substituted indolylthiazolidinones **30** – **33** against representative *Gram* positive and *Gram*-negative bacteria and fungi were tested by the disk diffusion method [35]. The results are listed in Table 4. From the data it is clear that compound **18** shows high activity against *Gram* positive bacteria, while compounds **19** – **29** possess moderate activity against *Gram* negative bacteria and fungi, compounds **30** – **33** possess more activity against fungi and moderate activity against *Gram* positive strains.

References

[1] M. Verma, V. R. Gujrati, M. S. Sharma, K. Anil, *et al.*, *Arch. Pharm*. **1982**, 358 – 363.

[2] A. Moll, H. Hübner, P. Gmeiner, R. Troschütz, *Bioorg. Med. Chem.* **2002**, *10*, 1671 – 1679.

[3] Y. Makato, S. Hiroaki, K. Harunhiko, H. Koichiro, Jpn. Kokai tokkyo koho **1992** Jp 03, 161, 470 [91, 161, 470] (Cl CO7D209/42). C. A. 116, 41299m.

[4] K. C. Joshi, V. N. Pathak, S. K. Jain, *J. Indian Chem. Soc*. **1980**, *LVII*, 1176 – 1980.

[5] A. M. Mahmoud, A. E. Abdel Rahman, G. M. Naggar, H. A. El-Sherief, *Indian J. Chem.* **1984**, *23B*, 379 – 381.

[6] K. M. Hussain, T. Shallendra, B. Kahkashan, *Indian J. Chem*. **1998**, *37B*, 1075 – 1077.

[7] K. C. Joshi, R. Jain, P. Chand, S. Gavg, *J. Indian Chem. Soc.* **1983**, *LX*, 760 – 761.

[8] M. Rajopadhye, F. D. Popp, *J. Heterocycl Chem*. **1984**, *21*, 289 – 291.

[9] A. A. El-Gendy, N. A. Abdou, E. Z. Sarhan, H. A. El-Banna, *Alex J. Pharm. Sci.* **1993**, *7*, 99 – 103.

[10] A. Monge, I. Aldana, E. Fernandez, *Eur. J. Med. (Chim. Ther.)* **1978**, *13*, 573 – 575.

[11] A. Monge, J. Palop, C. Gracia, E. Fernandez, *An. Real. Acad. Farm*. **1982**, *48*, 213 – 228.

[12] A. Monge, I. Aldana, M. Font, P. Parado, *et al.*, *An. Quim. Ser. C.* **1983**, *79*, 242 – 247.

[13] A. Monge, M. Font, P. Parrado, E. Fernandez, *Eur. J. Med. Chem*. **1988**, *23*, 547 – 552.

[14] A. Monge, I. Aldana, T. Alvarez, M. Losa, *et al.*, *Eur. J. Med. Chem*. **1991**, *26*, 655 – 658.

[15] N. N. Suvorov, Zh. D. Ovchinnikova, Yu. N. Sheinker, *Zh. Obshch. Khim*. **1961**, *31*, 2333 – 2339.

[16] A. Monge, I. Aldana, M. Losa, M. Font, E. Cenarruzabitia, *Arzneim. Forsch*. **1993**, *43,* 1175 – 1180.

[17] A. Monge, I. Aldana, M. Losa, M. Font, *et al.*, *J. Pharm. Sci*. **1993**, *82*, 526 – 530.

[18] A. Monge, M. Font, A. Cuartero, A. Elorriaga, *et al.*, *Eur. J. Med. Chem*. **1995**, *30*, 963 – 971.

[19] Heinrich Wieland, Egon Popper, H. Seefried, *Chem. Ber.* **1922**, *55,* 1816 – 1834.

[20] Y. Murakami, Y. Yokoyama, T. Mitura, H. Hirasawa, *et al.*, *Heterocycles* **1984**, *22*, 1211 – 1216.

[21] H. N. Rydon, J. C. Tweddle, *J. Chem. Soc*. **1955**, 3499 – 3503.

[22] E. Badger, S. Dicataldo, A. D. Kahle, J. Nadelson, M. J. Shapiro, *J. Heterocycl Chem.* **1981**, *18*, 623 – 626.

[23] A. Monge, E. Fernandez, *An. Quim.* **1972**, *68*, 1153.

[24] A. Monge, P. Parrado, M. Font, E. Fernandez, *J. Med. Chem*. **1987**, *30*, 1029 – 1035.

[25] T. Norgady, L. Morris, *Can. J. Chem*. **1969**, *47*, 1999 – 2002.

[26] A. Monge, I. Aldana, I. Lezamiz, E. Fernandez, *Synthesis* **1984**, *2*, 160 – 161.

[27] K. C. Joshi, R. Jain, S. Nishith, *J. Indian Chem. Soc*. **1991**, *68*, 625 – 627.

[28] S. R. El – Ezbawy, M. A. Alshaikh, *J. Indian Chem. Soc*. **1990**, *67*, 398 – 400.

[29] S. P. Lawande, B. R. Arbad, *J. Indian Chem. Soc*. **2000**, *77*, 352 – 354.

[30] B. Goel, T. Ram, R. Tyagi, E. Bansal, *et al.*, *Eur. J. Med. Chem*. **1999**, *34*, 265 – 269.

[31] A. Monge, I. Aldana, E. Fernandez, *J. Heterocylic Chem*. **1981**, *18*, 1533 – 1536.

[32] S. Swaminathan, K. Narisimhan, *Chem. Ber*. **1966**, *99*, 889 – 894.

[33] T. L. Kerley, A. C. Richard, R. W. Begley, B. E. Abery, L. C. Weaver, *J. Pharmacol. Exp. Ther.* **1961**, *132*, 360 – 365.

[34] P. Janssen, A. Jageneau, *J. Pharmacol. Exp. Ther*. **1960**, *129*, 471 – 475.

[35] A. W. Bauer, W. M. Kirby, J. C. Scherris, *Am. J. Clin. Pathol*. **1966**, *45*, 493 – 496.

[36] F. L. Allen, J. C. Brunton, H. Suschitzky, *J. Chem. Soc.* **1955**, 1283 – 1286.

[37] C. Frederick, P. Charles, *J. Chem. Soc*. **1915**, *107*, 32 – 34.

[38] H. Ishii, Y. Murakami, K. Hosoya, H. Takeda, *et al.*, *Chem. Pharm. Bull.* **1973**, *21*, 1481 – 1494.

[39] A. A. El-Gendy, A. F. Abou-Sier, *Egypt J. Pharm. Sci*. **1993**, *34*, 207 – 218.

[40] E. S. H. El Ashry, E. Ramadan, A. A. Kassem, M. Haggar, *Adv. Heterocycl. Chem.* **2005**, *88*, 1 – 110; E. S. H. El Ashry, E. Ramadan, A. A. Kassem, *Adv. Heterocycl. Chem.* **2006**, *90*, 1 – 127; E. S. H. El Ashry, E. Ramadan, A. A. Kassem, *Arkivoc* **2006**, *ix*, 1 – 15.

