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Synthesis of 1-acyl-2-alkylthio-1,2,4-triazolobenzimidazoles with antifungal, anti-inflammatory and analgesic effects

BAHAA G. MOHAMED
ABDEL-ALIM M. ABDEL-ALIM*
MOSTAFA A. HUSSEIN

Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut-71526, Egypt

Some new derivatives of 1,2,4-triazolo[2,3-a]benzimidazoles were synthesized through the reaction of 1,2-diaminobenzimidazole with carbon disulfide. The resulting 1,2,4-triazolo[2,3-a]benzimidazole-2-thione intermediate reacted with one equivalent of the alkyl halide to give the corresponding 2-alkylthio derivative **3a**-**g**. The latter were acylated to afford the 1-acyl-2-alkylthio--1,2,4-triazolo[2,3-a]-benzimidazole derivatives **4-10** in good yields. Structures of the new compounds were verified on the basis of spectral and elemental methods of analyses. Fourteen of the prepared compounds were tested for their possible antifungal activities. Most of the tested compounds showed activity against *Candida albicans and Fusarium oxysporum* comparable to that of fluconazole as a reference drug. Compounds **8a**, **9a**, and **10d** are the most active ones against most of the fungi used. Compounds **3e**, **4d**, **5d**, **6d**, **7d**, **8c**, **8d**, **9d**, and **10d** were tested for their anti-inflammatory and analgesic effects; most of these compounds showed potent and significant results compared to indomethacin. Moreover, ulcerogenicity and the median lethal dose (LD_{50}) of the most active compound **8d** were determined in mice; LD_{50} was found to be 275 mg kg^{-1} (*i.p.*).

Keywords: 1,2-diaminobenzimidazole, hydroxylamine-*O*-sulfonic acid, 1,2,4-triazolo-[2,3-a]benzimidazoles, antifungal, analgesic, anti-inflammatory activity

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The current literature indicates that benzimidazole derivatives possess diverse pharmacological activities, including antimicrobial (1-3), antiviral (4), antineoplastic (5), analgesic (6), anti-inflammatory (7, 8), antihypertensive (9), and vasodilating activities (10). Also, it is well-documented that triazole nucleus is associated with a variety of pharmacological actions. Triazoles display pronounced antimicrobial (11-13), antitubercular (14) and anti-inflammatory activities (15). Likewise, many fused heterocycles, including triazoles, were found to possess different pharmacological effects (16).

* Correspondence, e-mail: alim@acc.aun.edu.eg

Commercially available non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for reducing inflammation pain. The anti-inflammatory mechanism of NSAIDs is due to reduction of prostaglandin synthesis by the inhibition of cyclooxygenase isozymes (COX_1 and COX_2). Disruption of the cytoprotective prostaglandins by most of the currently used NSAIDs results in a mechanism-based toxicity, mainly in the gastrointestinal tract and kidney, which limits their therapeutic usefulness when long-term therapy is required (17). So far, various substituted 1,2,4-triazolothiones and some of their condensed derivatives have been approached as anti-inflammatory agents (17). Accordingly, the present work aims at the design and synthesis of new 2-alkyl-1,2,4-triazolobenzimidazoles (**3a-g**) and 1-acyl-2-alkyl-1,2,4-triazolobenzimidazoles (**4a-d**, **5a-d**, **6a-d**, **7a-d**, **8a-d**, **9a-d**, **10a-d**). Synthetic pathways include different chemical modifications of the structure of the parent compound **2**, which might modulate the physicochemical properties and consequently the biological activities of the target compounds. Moreover, the study includes testing the target compounds for their expected antifungal along with their analgesic-anti-inflammatory effects.

EXPERIMENTAL

Materials and equipment

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, model SMP1, UK) and were uncorrected. Precoated silica gel plates (Kieselgel 0.25 mm, 60G F254, Merck, Germany) were used for thin layer chromatography. The developing solvent system chloroform/methanol (9:1, *V/V*) was used (for compounds **4b**, **6d** and **10d**, a benzen/hexane mixture, 1:1, *V/V*). Spots were detected under UV (254 nm) (Spectroline, model CM-10, USA). Compounds **3a-g** and **4-10** were crystallized from ethanol unless otherwise specified.

IR spectra (KBr discs) were recorded on a Shimadzu IR-470 spectrometer (Shimadzu, Japan). ^{1}H NMR spectra were scanned on a Varian EM-360 L NMR spectrometer (60 MHz), (Varian, USA). Chemical shifts are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard, using $CDCl_3$, unless otherwise specified, as a solvent. Elemental analyses were performed at the Unit of Microanalysis, Assiut University (Assiut, Egypt).

Most of the chemicals used were of commercial grade: *o*-phenylenediamine (Aldrich, Germany), bromine (Aldrich), sodium cyanide, sodium hydroxide, potassium carbonate, potassium hydroxide, and gum acacia (El Nasr Pharm. Co., Egypt), hydroxylamine sulphate (Aldrich), alkyl halides (Fluka, Switzerland) carrageenan (Sigma, USA), carbon disulphide (Riedel-de Haën, Germany), and indomethacin (Nile Co., Egypt), Potato Dextrose Agar (PDA) or Sabouraud Agar (SA) media were prepared in the Department of Botany, Faculty of Science, Assiut University.

The starting material 1,2-diaminobenzimidazole **1** was prepared according to a reported procedure (18) through the reaction of *o*-phenylenediamine with cyanogen bromide (19), followed by amination using hydroxylamine-*O*-sulfonic acid (Scheme 1).

Synthesis of 1,2,4-triazolo[2,3-a]benzimidazole-2-thione

To a stirred solution of 1,2 diaminobenzimidazole (10.0 g, 0.052 mol) in DMF (100 mL), CS_2 (30 mL, 0.47 mol) was added (Scheme 1). The reaction mixture was refluxed for 16 h and the formed precipitate was filtered, washed with methanol and dried. The product is insoluble in most organic solvents; hence, it was purified by dissolving in 5% KOH. The alkaline solution was cooled in an ice bath, and then rendered acidic by addition of conc. HCl under stirring. The precipitate was filtered, washed with distilled water and dried. The process of purification was repeated until constant melting point (Fig. 1, Table I).

*Synthesis of 2-alkylthio-1,2,4-triazolo[2,3-a]benzimidazoles (**3a–f**)*

To a suspension of compound **2** (9.5 g, 0.05 mol) and potassium carbonate (6.9 g, 0.05 mol) in dry acetone (100 mL), the appropriate alkyl halide (0.05 mol) was added. The reaction mixture was stirred for 10-12 h at ambient temperature. Acetone was evaporated, the residue was triturated with water, and then extracted with chloroform (3 × 15 mL). The chloroform extract was washed with water and dried with anhydrous magnesium sulfate. Chloroform was evaporated and the residue was crystallized from the appropriate solvent (Fig. 1, Tables I and II).

*Synthesis of 2-phenacyl-1,2,4-triazolo[2,3-a]benzimidazole (**3g**)*

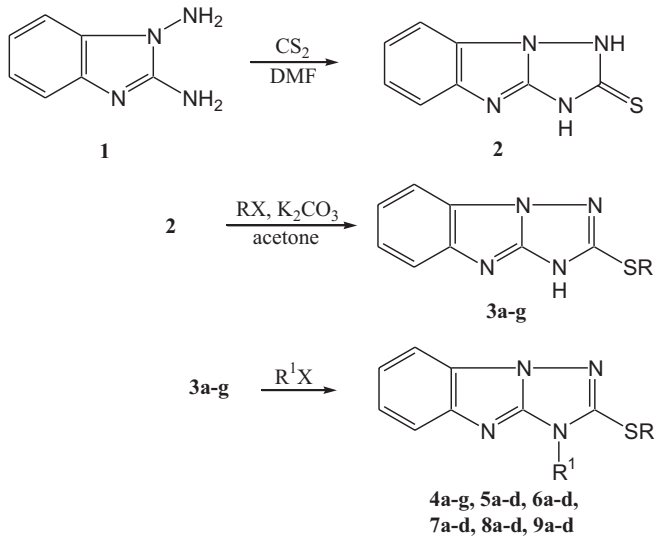
A mixture of compound **2** (9.5 g, 0.05 mol) and a solution of phenacyl bromide (13.7 g, 0.07 mol) in dry ethanol (100 mL) was refluxed for 3 h in the presence of fused sodium acetate (4.1 g, 0.05 mol). The separated solid was filtered, washed with water and dried. The crude product was crystallized from ethanol as white crystals (Fig. 1, Table I).

*Synthesis of 1-acetyl(tosyl)-2-alkylthio-1,2,4-triazolo[2,3-a]benzimidazoles (**4a**,**d**, **5a**,**d**, **6a**,**d**, **7a**,**d**, **8a**,**d**, **9a**,**d**, **10a**,**d**)*

Acetyl or tosyl chloride (0.005 mol) was added to a suspension of the appropriate 2-alkylthio-1,2,4-triazolo[2,3-a]benzimidazole (**3a–g**) (0.005 mol) and anhydrous potassium carbonate (0.69 g, 0.005 mol) in dry acetone (15 mL). The reaction mixture was stirred for 6-8 h at ambient temperature and acetone was then evaporated. Distilled water was added to the residue and the formed precipitate was filtered, washed with water, dried and crystallized from the appropriate solvent (Fig. 1, Table I).

*Synthesis of 1-benzoyl(*p*-chlorobenzoyl)-2-alkylthio-1,2,4-triazolo [2,3-a]-benzimidazoles (**4b**,**c**, **5b**,**c**, **6b**,**c**, **7b**,**c**, **8b**,**c**, **9b**,**c**, **10b**,**c**)*

Benzoyl chloride or *p*-chlorobenzoyl chloride (0.004 mol) was dropped into a stirred and ice-cooled solution of 2-alkylthio 1,2,4-triazolo[2,3-a]benzimidazole (**3a–g**) (0.004 mol) and sodium hydroxide (0.2 g, 0.005 mol) in water (15 mL). The reaction mixture was stirred for 1 h. The separated solid was filtered, washed with water and dried. The crude products were then crystallized from the appropriate solvents (Scheme 1, Table I).



R = H, CH_3, C_2H_5, CH_2=$CHCH_2$, *n*-C_3H_7, *i*-C_3H_7, $C_6H_5CH_2$, $CH_2COC_6H_5$

R^1 = H, CH_3CO, C_6H_5CO, *p*-ClC_6H_4CO, *p*-$CH_3C_6H_4SO_2$

Table I. Physicochemical constants of compounds ***2-10***

Compd. No.	R	R^1	Yield (%)	M.p. (°C)[a]	Mol. formula (M_r)	Elemental analysis Calcd./found (%)		
						C	H	N
2	H	H	80	318–322	$C_8H_6N_4S$ (190.23)	50.51 50.02	3.18 3.05	29.45 29.27
3a	CH_3	H	68	143–145	$C_9H_8N_4S$ (204.25)	52.92 52.57	3.95 3.95	27.43 26.95
3b	C_2H_5	H	71	123–124	$C_{10}H_{10}N_4S$ (218.28)	55.02 54.54	4.62 4.64	25.67 25.49
3c	CH_2=$CHCH_2$	H	68	101–102	$C_{11}H_{10}N_4S$ (230.29)	57.37 57.25	4.38 4.28	24.33 24.19
3d	*n*-C_3H_7	H	72	158–159	$C_{11}H_{12}N_4S$ (232.31)	56.87 56.38	5.21 5.41	24.12 23.99
3e	$CH(CH_3)_2$	H	69	155–156	$C_{11}H_{12}N_4S$ (232.31)	56.87 56.87	5.21 4.69	24.12 23.82
3f	$C_6H_5CH_2$	H	77	118–120	$C_{15}H_{12}N_4S$ (280.35)	64.26 63.93	4.31 4.23	19.98 19.67

Table I contd.

Compd. No.	R	R^1	Yield (%)	M.p. (°C)[a]	Mol. formula (M_r)	Elemental analysis Calcd./found (%)		
						C	H	N
3g	$C_6H_5COCH_2$	H	90	121–123	$C_{16}H_{12}N_4OS$ (308.36)	62.32 61.85	3.92 3.82	18.17 17.90
4a	CH_3	CH_3CO	63	213–215	$C_{11}H_{10}N_4OS$ (246.29)	53.64 53.63	4.09 3.78	22.75 22.54
4b	CH_3	*p*-$CH_3C_6H_4SO_2$	85	214–216	$C_{16}H_{14}N_4O_2S_2$ (358.44)	53.61 53.85	3.94 3.64	15.63 15.65
4c	CH_3	C_6H_5CO	71	219–221	$C_{16}H_{12}N_4OS$ (308.36)	62.32 62.47	3.92 3.77	18.17 18.22
4d	CH_3	*p*-ClC_6H_4CO	86	211–212	$C_{16}H_{11}ClN_4OS$ (342.80)	56.06 56.02	3.23 2.72	16.34 16.48
5a	C_2H_5	CH_3CO	68	213–215	$C_{12}H_{12}N_4OS$ (260.32)	55.37 55.34	4.65 4.77	21.52 21.25
5b	C_2H_5	*p*-$CH_3C_6H_4SO_2$	85	222–223	$C_{17}H_{16}N_4O_2S_2$ (372.47)	54.82 54.42	4.33 4.54	15.04 15.08
5c	C_2H_5	C_6H_5CO	70	219–221	$C_{17}H_{14}N_4OS$ (322.39)	63.33 63.60	4.38 4.54	17.38 17.61
5d	C_2H_5	*p*-ClC_6H_4CO	84	189–191	$C_{17}H_{13}ClN_4OS$ (356.83)	57.22 56.70	3.67 3.29	15.70 15.42
6a	CH_2=$CHCH_2$	CH_3CO	81	167–168	$C_{13}H_{12}N_4OS$ (272.33)	57.34 57.34	4.44 4.19	20.57 20.57
6b	CH_2=$CHCH_2$	*p*-$CH_3C_6H_4SO_2$	82	183–185	$C_{18}H_{16}N_4O_2S_2$ (384.48)	56.23 55.75	4.19 4.23	14.57 14.82
6c	CH_2=$CHCH_2$	C_6H_5CO	85	175–175	$C_{18}H_{14}N_4OS$ (334.40)	64.65 64.68	4.22 4.26	16.75 16.87
6d	CH_2=$CHCH_2$	*p*-ClC_6H_4CO	86	161–162	$C_{18}H_{13}ClN_4OS$ (368.84)	58.61 58.90	3.55 3.53	15.19 15.46
7a	*n*-C_3H_7	CH_3CO	71	213–215	$C_{13}H_{14}N_4OS$ (274.34)	56.91 56.86	5.14 5.04	20.42 20.40
7b	*n*-C_3H_7	*p*-$CH_3C_6H_4SO_2$	85	143–145	$C_{18}H_{18}N_4O_2S_2$ (386.49)	55.94 55.59	4.69 5.05	14.50 14.43
7c	*n*-C_3H_7	C_6H_5CO	85	219–221	$C_{18}H_{15}N_4OS$ (336.41)	64.26 63.79	4.79 4.99	16.65 16.72
7d	*n*-C_3H_7	*p*-ClC_6H_4CO	84	198–199	$C_{18}H_{16}ClN_4OS$ (370.86)	58.30 58.38	4.08 3.78	15.11 15.14
8a	$CH(CH_3)_2$	CH_3CO	68	123–125	$C_{13}H_{14}N_4OS$ (274.34)	56.91 57.09	5.14 5.11	20.42 20.67
8b	$CH(CH_3)_2$	*p*-$CH_3C_6H_4SO_2$	85	212–213	$C_{18}H_{18}N_4O_2S_2$ (386.49)	55.94 55.53	4.69 4.94	14.50 14.66

Table I contd.

Compd. No.	R	R^1	Yield (%)	M.p. (°C)[a]	Mol. formula (M_r)	Elemental analysis Calcd./found (%)		
						C	H	N
8c	$CH(CH_3)_2$	C_6H_5CO	75	194–195	$C_{18}H_{16}N_4OS$ (336.41)	64.26 64.28	4.79 5.34	16.65 16.86
8d	$CH(CH_3)_2$	*p*-ClC_6H_4CO	85	202–203	$C_{18}H_{15}ClN_4OS$ (370.86)	58.30 57.81	4.08 3.75	15.11 15.03
9a	$C_6H_5CH_2$	CH_3CO	78	128–130	$C_{17}H_{14}N_4OS$ (322.39)	63.33 63.14	4.38 3.97	17.38 17.75
9b	$C_6H_5CH_2$	*p*-$CH_3C_6H_4SO_2$	85	208 (dec.)	$C_{22}H_{18}N_4O_2S_2$ (434.54)	60.81 60.96	4.18 4.02	12.89 12.86
9c	$C_6H_5CH_2$	C_6H_5CO	80	197–198	$C_{22}H_{16}N_4OS$ (384.45)	68.73 68.62	4.19 3.71	14.57 14.50
9d	$C_6H_5CH_2$	*p*-ClC_6H_4CO	84	194–195	$C_{22}H_{15}ClN_4OS$ (418.90)	63.08 63.12	3.61 3.08	13.37 13.63
10a	$C_6H_5COCH_2$	CH_3CO	75	191–193	$C_{18}H_{14}N_4O_2S$ (350.40)	61.70 61.78	4.03 3.87	15.99 16.09
10b	$C_6H_5COCH_2$	*p*-$CH_3C_6H_4SO_2$	85	206–207	$C_{23}H_{18}N_4O_3S_2$ (462.55)	59.72 59.50	3.92 3.95	12.11 11.92
10c	$C_6H_5COCH_2$	C_6H_5CO	82	196–198	$C_{23}H_{16}N_4O_2S$ (412.46)	66.97 66.77	3.91 3.47	13.58 13.78
10d	$C_6H_5COCH_2$	*p*-ClC_6H_4CO	86	187–189	$C_{23}H_{15}ClN_4O_2S$ (446.91)	61.81 62.14	3.38 3.00	12.54 12.17

[a] Compounds **3b, 3c,** and **5d** crystallized from acetone, compounds **3d, 5b, 8b,** and **10b** from methanol, and compounds **3f, 4c, 5c, 6c, 7c, 7d, 8c,** and **9c** from petrol ether.

Antifungal activity

The synthesized compounds **2**, **3b** and **4-10** were tested for their antifungal activity *in vitro*, in comparison with fluconazole as a reference drug using the standard agar disk diffusion method (20) against six pathogenic, phytopathogenic or food poisoning fungal species *Trichophyton rubrum* (Castellani) Sabouraud (AUMC 405), *Candida albicans* (Robin) Berkhout (AUMC 418), *Microsporum canis* Gruby (AUMC 241), *Aspergillus flavus* Thom (AUMC 30), *Fusarium oxysporum* Schlechtendal (AUMC 210), and *Penicillium expansum* Link (AUMC 525). Fungal cultures were obtained from the Assuit University Mycological Center (AUMC, Assiut, Egypt).

A spore suspension in sterile distilled water was prepared from 2-5 days old culture of the test fungi growing on Potato Dextrose Agar (PDA) or Sabouraud Agar (SA) me-

dia. The final spore concentration was 5×10^4 spores mL^{-1}. About 15 mL of the growth medium was placed into sterilized Petri dishes of 9 cm diameter and inoculated with 1 mL of the spore suspension. Plates were shaken gently to homogenize the inocula.

Sterile 5-mm filter paper disk (Whatman, UK) was saturated with 10 μL of the test compound solution or fluconazole as a reference (100 μmol L^{-1} in DMSO) or DMSO as negative control. Impregnated disks were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 28 ± 2 °C for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured and the results are given in Table III.

Anti-inflammatory and analgesic activity

Animals were housed in separate cages, 6 animals each, in temperature-controlled rooms at 25 °C. Animals were allowed free access to food and water and maintained at a 12 h light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animals use and care as found in the European Community Guidelines.

For determination of anti-inflmmatory and analgesic activities, all test compounds and reference drugs were suspended in 5% solution of gum acacia in normal saline. Suspensions of the test compounds, reference drugs and 5% gum acacia-saline solution (negative control) were injected 1 mL each *i.p.*

The anti-inflammatory activity of nine representatives of the synthesized compounds (**3e**, **4d**-**10d** and **8c**) was evaluated according to the method described by Winter *et al*. (21), where a pedal inflammation in rat paws was induced by subplantar injection of 0.2 mL carrageenan (0.2%) suspension in gum acacia into the right hind of the rats.

Male adult albino rats (100–120 g) were divided into groups, 11 groups altogether, of five animals each. The rat paw thickness was measured with a Veriner caliper (SMIEC, China) before and 1 h after the carrageenan injection to detect the carrageenan induced inflammation. Each test compound at a dose of 10 mg kg^{-1} was injected *i.p.* to a separate group of rats 1 h after carrageenan injection. Control group received the vehicle (5% gum acacia), while the reference group received indomethacin, 10 mg kg^{-1}.

The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at zero, 1, 2, 3 and 5 h after injection of the test compounds, reference drug, and the vehicle. The results of anti-inflammatory activity of the test compounds and the reference drug are listed in Table IV.

The analgesic activity of compounds (**3e**, **4d**, **5d**, **6d**, **7d**, **8c**, **8d**, **9d** and **10d**) was determined in mice using the hot plate method (22) in comparison with indomethacin. In this method, the time taken by the mouse to lick its feet or to jump within the Plexiglas cylinder placed on a hot plate surface (55 °C) was determined. This reaction time was taken as the end point and the increase in hot plate latency was taken as the measure of analgesic activity.

Male adult albino mice (20-25 g) were divided into eleven groups, of five animals each. Nine test compounds and the reference drug were injected *i.p.* into mice at a dose of 10 mg kg^{-1}. Control group of animals was similarly treated with 5% gum acacia. The reaction time was evaluated directly 0.5, 1, 2, 3 and 5 h of first injection. The results of analgesic activity of the test compounds and indomethacin are listed in Table V.

Gastric ulceration

Gastric lesions on the mucosa were determined using a Stereoscopic Microscope (XSC-07, China). Observation of the gastrointestinal mucosa for the presence of lesions following oral administration of graded doses of the test compounds as well as the reference drug was taken as an indication of ulcerogenic effects. Both the frequency of ulceration (expressed as the ratio of ulcerated animals) and the severity of ulceration (expressed as ulcer index) were used for comparison of the tested compounds and indomethacin (23).

Six groups, of six male adult albino mice each, were fasted for 24 h. The tested compounds and indomethacin were administered orally in doses of 10, 30 and 50 mg kg^{-1}, as suspensions in 5% gum acacia saline solution. After 6 h, the animals were killed, the stomachs were removed and gastric lesions on the mucosa were determined using a stereoscopic microscope. Ulcer was defined as at least one lesion 0.5 mm or more in length. All lesions of more than 0.1 mm in length were summed to obtain the ulcer index and the results were given in Table VI.

Determination of acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of compound **8d** was determined in mice. Groups of male adult albino mice, of five animals (25–30 g) each, were injected *i.p.* with graded doses of the test compound. The percentage of mortality in each group of animals was determined 72 h after the injection. Computation of LD_{50} was processed by a graphical method (24).

RESULTS AND DISCUSSION

Chemistry

The starting material 1,2-diaminobenzimidazole (**1**) was prepared according to a reported procedure through the reaction of *o*-phenylenediamine with cyanogen bromide (18), followed by amination using hydroxylamine-*O*-sulfonic acid (19). Structure of compound (**1**) was confirmed by comparison of its physical and spectral data with the reported ones (19). ^{1}H NMR spectrum of compound **1** showed two broad singlets at δ 5.48 and 6.08 (two amino groups) besides a multiplet at δ 7.30–8.10, equivalent to the four aromatic protons as reported (19).

The key intermediate 1,2,4-triazolo[2,3-a]benzimidazole-2-thione (**2**) was prepared by the interaction of 1,2-diaminobenzimidazole with CS_2 in DMF (Fig. 1 and Table 1). Structure of compound **2** was confirmed from its IR, ^{1}H NMR as well as elemental analysis. IR spectrum showed a broad band at 3410 cm^{-1} (NH). ^{1}H NMR spectrum revealed the presence of a broad singlet at δ 12.50 ppm (two NH groups) exchangeable with D_2O and a multiplet at δ 7.33–8.13 corresponding to the four aromatic protons. MS spectrum of compound **2** revealed the molecular ion peak M^+ at *m/z* 190 (100%), corresponding to the molecular mass of this compound. Also, the spectrum showed prominent peaks at *m/z* 132 (66.2%) and *m/z* 90 (50.7%).

2-Alkylthio-1,2,4-triazolo[2,3-a]benzimidazole derivatives **3a-g** were synthesized by the reaction of 1,2,4-triazolo[2,3-a]benzimidazole-2-thione (**2**) with one equivalent of respective alkyl halide (Fig. 1 and Table I). The reaction afforded the *S*-alkyl derivatives since the *S*-alkylation supersedes the *N*-alkylation due to higher nucleophilicity and polarizability of the SH group rather than the NH one (25, 26). Structures of compounds **3a-g** were confirmed by elemental analyses, IR and ^{1}H NMR spectral data. IR spectra of compounds **3a-g** showed a broad band at 3410–3420 cm^{-1} (NH stretching) while their ^{1}H NMR spectra showed a broad singlet at δ 12.00–13.00 ppm corresponding to NH, which is exchangeable with D_2O. Moreover, the *S*-alkyl substituents gave a pattern in the ^{1}H NMR spectra in accordance with the expected structures of these compounds (Table II).

*Table II. ^{1}H NMR data of compounds **2**–**10***

Compd. No.	R	R^1	^{1}H NMR ($CDCl_3$, δ ppm)
2[a]	H	H	7.33–8.16 (m, 4H, C_6H_4), 12.00 (hump, 2H, 2NH)
3a[a]	CH_3	H	2.75 (s, 3H, SCH_3), 5.58 (hump, 1H, NH), 7.26–8.01 (m, 4H, C_6H_4)
3b	C_2H_5	H	1.45 (t, 3H, SCH_2CH_3), 2.90–3.58 (q, 2H, SCH_2CH_3), 7.20–8.00 (m, 4H, C_6H_4), 12.00 (hump, 1H, NH)
3c	CH_2=$CHCH_2$	H	3.87 (d, 2H, SCH_2CH=CH_2, *J* = 8.50 Hz); 5.00–5.58 (m, 2H, SCH_2CH=CH_2), 5.83–6.63 (m, 1H, SCH_2CH=CH_2), 7.25–8.00 (m, 4H, C_6H_4), 12.50 (hump, 1H, NH)
3d	*n*-C_3H_7	H	1.07 (t, 3H, $SCH_2CH_2CH_3$); 1.60–2.21 (m, 2H, $SCH_2CH_2CH_3$), 3.26 (t, 2H, $SCH_2CH_2CH_3$), 7.26–8.03 (m, 4H, C_6H_4), 12.50 (hump, 1H, NH)
3e	$CH(CH_3)_2$	H	1.53 (d, 6H, $SCH(CH_3)_2$, *J* = 8.50 Hz), 3.66–4.30 (m, 1H, $SCH(CH_3)_2$), 7.30–8.00 (m, 4H, C_6H_4), 12.50 (hump, 1H, NH)
3f	$C_6H_5CH_2$	H	4.56 (s, 2H, SCH_2Ph), 7.16–8.00 (m, 9H, C_6H_4, $C_6H_5CH_2$), 13.00 (hump, 1H, NH)
3g	$C_6H_5COCH_2$	H	4.92 (s, 2H, SCH_2COPh), 7.3–8.42 (m, 9H, C_6H_4, $C_6H_5COCH_2$), 12.36 (hump, 1H, NH)
4a	CH_3	CH_3CO	2.70 (s, 3H, SCH_3), 2.95 (s, 3H, $NCOCH_3$), 7.30–8.84 (m, 4H, C_6H_4)
4b	CH_3	*p*-$CH_3C_6H_4SO_2$	2.46 (s, 3H, *p*-$CH_3C_6H_4SO_2$), 2.80 (s, 3H, SCH_3), 7.30–8.55 (m, 8H, C_6H_4 and *p*-$CH_3C_6H_4SO_2$)
4c	CH_3	C_6H_5CO	2.61 (s, 3H, SCH_3), 7.40-8.65 (m, 9H, C_6H_4, COC_6H_5)
4d	CH_3	*p*-ClC_6H_4CO	2.60 (s, 3H, SCH_3), 7.30–8.30 (m, 8H, C_6H_4, $NCOC_6H_4Cl$)

Table II contd.

Compd. No.	R	R^1	^{1}H NMR ($CDCl_3$, δ ppm)
5a	C_2H_5	CH_3CO	1.48 (t, 3H, SCH_2CH_3), 3.00 (s, 3H, $NCOCH_3$), 3.06–3.53 (q, 2H, SCH_2CH_3), 7.30–8.00 (m, 4H, C_6H_4), 8.40–8.78 (m, 1H, C_6H_4)
5b	C_2H_5	*p*-$CH_3C_6H_4SO_2$	1.50 (t, 3H, SCH_2CH_3), 2.41 (s, 3H, CH_3), 3.05–3.63 (q, 2H, *p*-$CH_3C_6H_4SO_2$), 7.11–8.50 (m, 8H, C_6H_4, *p*-$CH_3C_6H_4SO_2$)
5c	C_2H_5	C_6H_5CO	1.38 (t, 3H, SCH_2CH_3), 2.84-3.33 (q, 2H, SCH_2CH_3), 7.16–8.66 (m, 9H, C_6H_4, COC_6H_5)
5d	C_2H_5	*p*-ClC_6H_4CO	1.33 (t, 3H, SCH_2CH_3), 2.85–3.33 (q, 2H, SCH_2CH_3), 7.31–8.63 (m, 8H, C_6H_4, COC_6H_4Cl)
6a	CH_2=$CHCH_2$	CH_3CO	3.00 (s, 3H, $NCOCH_3$), 3.92 (d, 2H, SCH_2CH=CH_2, *J* = 8.50 Hz), 5.07–5.70 (m, 2H, SCH_2CH=CH_2), 5.82–6.47 (m, 1H, SCH_2CH=CH_2), 7.24–7.94 (m, 4H, C_6H_4), 8.34–8.82 (m, 1H, C_6H_4)
6b	CH_2=$CHCH_2$	*p*-$CH_3C_6H_4SO_2$	2.42 (s, 3H, CH_3), 3.94 (d, 2H, SCH_2CH=CH_2, *J* = 8.50 Hz), 5.00–5.76 (m, 2H, SCH_2CH=CH_2), 5.85–6.57 (m, 1H, SCH_2CH=CH_2), 7.20–8.58 (m, 8H, C_6H_4, *p*-$CH_3C_6H_4SO_2$)
6c	CH_2=$CHCH_2$	C_6H_5CO	3.70 (d, 2H, SCH_2CH=CH_2, *J* = 8.50 Hz), 4.74–5.58 (m, 2H, SCH_2CH=CH_2), 5.72–6.44 (m, 1H, SCH_2CH=CH_2), 7.12–8.74 (m, 9H, C_6H_4, COC_6H_5)
6d	CH_2=$CHCH_2$	*p*-ClC_6H_4CO	3.78 (d, 2H, SCH_2CH=CH_2, *J* = 8.50 Hz), 5.00–5.55 (m, 2H, SCH_2CH=CH_2), 5.74–6.50 (m, 1H, SCH_2CH=CH_2), 7.38–8.74 (m, 8H, C_6H_4, COC_6H_4Cl)
7a	*n*-C_3H_7	CH_3CO	1.12 (t, 3H, $SCH_2CH_2CH_3$), 1.50–2.22 (m, 2H, $SCH_2CH_2CH_3$), 2.60–3.54 (m, 5H, $NCOCH_3$, $SCH_2CH_2CH_3$), 7.12–8.74 (m, 4H, C_6H_4)
7b	*n*-C_3H_7	*p*-$CH_3C_6H_4SO_2$	1.13 (t, 3H, $SCH_2CH_2CH_3$), 1.52–2.32 (m, 2H, $SCH_2CH_2CH_3$), 2.45 (s, 3H, CH_3), 3.34 (t, 2H, $SCH_2CH_2CH_3$), 7.24–8.64 (m, 8H, C_6H_4, *p*-$CH_3C_6H_4SO_2$)
7c	*n*-C_3H_7	C_6H_5CO	1.00 (t, 3H, $SCH_2CH_2CH_3$), 1.35–2.15 (m, 2H, $SCH_2CH_2CH_3$), 3.06 (t, 2H, $SCH_2CH_2CH_3$), 7.13–8.67 (m, 9H, C_6H_4, COC_6H_5)
7d	*n*-C_3H_7	*p*-ClC_6H_4CO	1.00 (t, 3H, $SCH_2CH_2CH_3$), 1.50–2.17 (m, 2H, $SCH_2CH_2CH_3$), 3.67 (t, 2H, $SCH_2CH_2CH_3$), 7.33–8.14 (m, 7H, C_6H_4, COC_6H_4Cl), 8.34–8.74 (m, 1H, C_6H_4, COC_6H_4Cl)
8a	$CH(CH_3)_2$	CH_3CO	1.50 (d, 6H, $SCH(CH_3)_2$ *J* = 8.50 Hz), 2.95 (s, 3H, $NCOCH_3$), 3.64–4.27 (m, 1H, $SCH(CH_3)_2$), 7.25–8.00 (m, 3H, C_6H_4), 8.37–8.74 (m, 1H, C_6H_4)

Table II contd.

Compd. No.	R	R^1	^{1}H NMR ($CDCl_3$, δ ppm)
8b	$CH(CH_3)_2$	*p*-$CH_3C_6H_4SO_2$	1.50 (d, 6H, $SCH(CH_3)_2$, *J* = 8.50 Hz), 2.44 (s, 3H, CH_3), 3.68–4.47 (m, 1H, $SCH(CH_3)_2$), 7.15–8.68 (m, 8H, C_6H_4, *p*-$CH_3C_6H_4SO_2$)
8c	$CH(CH_3)_2$	C_6H_5CO	1.37 (d, 6H, $SCH(CH_3)_2$, *J* = 8.50 Hz), 3.38–4.10 (m, 1H, $SCH(CH_3)_2$), 7.18–8.74 (m, 9H, C_6H_4, COC_6H_5)
8d	$CH(CH_3)_2$	*p*-ClC_6H_4CO	1.35 (d, 6H, $SCH(CH_3)_2$, *J* = 8.50 Hz), 3.37–4.00 (m, 1H, $SCH(CH_3)_2$), 7.30–8.14 (m, 7H, C_6H_4, COC_6H_4Cl), 8.30–8.64 (m, 1H, C_6H_4, COC_6H_4Cl)
9a	$C_6H_5CH_2$	CH_3CO	2.95 (s, 3H, $NCOCH_3$), 4.53 (s, 2H, SCH_2Ph), 6.95–8.00 (m, 8H, C_6H_4, $SCH_2C_6H_5$), 8.05–8.76 (m, 1H, C_6H_4, $SCH_2C_6H_5$)
9b	$C_6H_5CH_2$	*p*-$CH_3C_6H_4SO_2$	2.45 (s, 3H, *p*-$CH_3C_6H_4SO_2$), 4.60 (s, 2H, SCH_2Ph), 7.05–8.58 (m, 13H, C_6H_4, *p*-$CH_3C_6H_4SO_2$, $SCH_2C_6H_5$)
9c	$C_6H_5CH_2$	C_6H_5CO	4.64 (s, 2H, SCH_2Ph), 7.18–8.22 (m, 14H, C_6H_4, $SCH_2C_6H_5$, $NCOC_6H_5$)
9d	$C_6H_5CH_2$	*p*-ClC_6H_4CO	4.30 (s, 2H, SCH_2Ph), 7.36–8.64 (m, 13H, C_6H_4, $SCH_2C_6H_5$, $NCOC_6H_4Cl$)
10a	$C_6H_5COCH_2$	CH_3CO	2.83 (s, 3H, $NCOCH_3$), 4.83 (s, 2H, SCH_2COPh), 7.10–8.65 (m, 9H, C_6H_4, SCH_2CO C_6H_5)
10b	$C_6H_5COCH_2$	*p*-$CH_3C_6H_4SO_2$	2.38 (s, 3H, *p*-$CH_3C_6H_4SO_2$), 4.87 (s, 2H, SCH_2COPh), 7.15–8.45 (m, 13H, C_6H_4, *p*-$CH_3C_6H_4SO_2$, SCH_2CO C_6H_5)
10c	$C_6H_5COCH_2$	C_6H_5CO	4.64 (s, 2H, SCH_2COPh), 7.17–8.30 (m, 14H, C_6H_4, SCH_2CO C_6H_5, $NCOC_6H_5$)
10d	$C_6H_5COCH_2$	*p*-ClC_6H_4CO	4.86 (s, 2H, SCH_2COPh), 7.20–8.15 (m, 13H, C_6H_4, SCH_2CO C_6H_5, $NCOC_6H_4Cl$)

[a] Spectra were taken in DMSO-d_6.

On the other hand, reaction of compounds **3a-g** with one of the different acyl halides in the presence of either 5% aqueous NaOH solution (Schotten-Baumann reaction) (27) or K_2CO_3 and acetone afforded 1-acyl-2-alkylthio-1,2,4-traizolo[2,3-a]benzimidazole derivatives (**4a**,**d**, **5a**,**d**, **6a**,**d**, **7a**,**d**, **8a**,**d**, **9a**,**d**, and **10a**,**d**). The physicochemical properties of these compounds are given in Table I. Structures of compounds (**4a**,**d**, **5a**,**d**, **6a**,**d**, **7a**,**d**, **8a**,**d**, **9a**,**d**, and **10a**,**d**) were established by IR and ^{1}H NMR spectrometries as well as elemental analyses. In IR spectra, disappearance of the absorption band at 3410–3420 cm^{-1}, along with the appearance of a strong absorption band corresponding to the carbonyl group (1680–1710 cm^{-1}) confirmed the *N*-acylation. Also, disappearance of the exchangeable broad singlet in the ^{1}H NMR spectra is taken as an evidence for introduction of the acyl group (Fig. 1). MS of **5d** revealed the molecular ion peak M^+ at *m/z*

356 (29.2%) corresponding to the molecular mass of this compound; CH ^{35}ClNOS and a peak at M^++2 at (*m/z* 358, 12.5%) corresponding to CH ^{37}ClNOS.

Antifungal activity

Results of antifungal activity are given in Table III. These results indicate that most of the tested compounds were active against *Candida albicans* and their activity ranged between 30 and 80% of that of fluconazole. Also, about half of the tested compounds showed 35–94% of the antifungal activity of the reference drug against *Fusarium oxysporum*. On the other hand, compounds **6d**, **8a, 9a,** and **10d** exhibited 70-80% of the antifungal activity of fluconazole against *Microsporium canis*, and about 37% of the activity of the drug against *Trichophyton rubrum*.

It is noteworthy that fluconazole was inactive against *Aspergillus flavus* and *Penicillium expansum*, while compounds **4d**, **6a**, **8a**, and **9a** were active. These results can be explained in view of the reported data about the antifungal activity of azoles (28), which indicate that they are active against dermatophytes such as *Trichophyton*, *Epidermophyton*, and *Microsporum* spp., and yeasts such as *Candida albicans*. On the other hand, no data

Table III. Antifungal activity of test compounds

Compd.	Inhibition zone (mm)					
	Fungus					
	Candida albicans	*Trichophyton rubrum*	*Microsporium canis*	*Aspergillus flavus*	*Penicillium expansum*	*Fusarium oxysporum*
DMSO	–	–	–	–	–	–
Fluconazole[a]	20	18	10	–	–	17
2[a]	8	–	–	–	–	10
3b[a]	10	–	–	–	–	14
4a[a]	10	–	–	–	–	–
4d[a]	9	–	–	–	12	–
5a[a]	7	–	–	–	–	10
5d[a]	–	–	–	–	–	16
6a[a]	6	–	0	7	6	–
6d[a]	10	6	7	–	–	–
7a[a]	–	–	–	–	–	–
7d[a]	–	–	–	–	–	–
8a[a]	10	8	7	9	7	7
8d[a]	12	–	–	–	–	8
9a[a]	16	6	8	8	7	6
9d[a]	9	–	–	–	–	–
10a[a]	–	–	–	–	–	12
10d[a]	9	7	7	–	7	10

[a] c = 1 μmol L^{-1}.

are mentioned in the available literature about their activity against *Aspergillus flavus* and *Penicillium expansum*. The difference in the obtained antifungal activity between the tested compounds and fluconazole might be attributed to their different three-dimentional structures. This difference may affect their selectivity against the fungal cytochrome P-450 enzymes being responsible for the growth of fungi (28).

Anti-inflammatory and analgetic activity

Nine compounds **3e**, **4d**, **5d**, **6d**, **7d**, **8c**, **8d**, **9d**, and **10d**, were selected for these tests on the ground that seven of them contain the *p*-chlorobenzoyl substituent at N1 and the other 2 contain either H or benzoyl moieties at N1 in order to investigate the crucial role of the *p*-chlorobenzoyl substituent on anti-inflammatory and analgesic activities. Previous results were reported by Black *et al.* (29) and Kalgutkar *et al.* (30) indicating the vital role of the *p*-chlorobenzoyl substituent for the activity of indomethacin and similar compounds (cf. the activity of **3e** and **8c**). The tested compounds could be considered as 3-aza-indolotriazole derivatives and consequently we expected them to induce anti-inflammatory and analgesic effects.

The results for compounds **3e**, **4d**, **5d**, **6d**, **7d**, **8d**, **8d**, **9d**, and **10d**, are presented in Table IV, which revealed that within a 3 h interval most of the test compounds exhibited anti-inflammatory activity comparable to that of indomethacin. On the other hand, only compounds **6d**, **7d**, and **8d** exhibited almost comparable results to those of indomethacin 5 h after injecting them, whereas compounds **9d** and **10d** showed only 85% and 85.9% of the activity of indomethacin. The delayed effect of compounds **6d**-**8d** compared to **4d** and **5d** can be explained as follows:

Table IV. Inhibitory effects of test compounds upon carrageenan induced paw edema in rats

Compound	Edema inhibition (%)				
	Time after compound injection (h)				
	0	1	2	3	5
5% gum acacia	–	–	–	–	–
Indomethacin[a]	7.16	23.50	43.32	66.16	92.25
3e[a]	1.72	13.50	35.51	38.34	46.00
4d[a]	1.97	6.25	35.76	63.65	43.00
5d[a]	0.24	16.75	50.12	60.40	34.00
6d[a]	0.98	13.50	48.11	63.40	90.80
7d[a]	0.24	18.00	37.53	62.90	90.50
8c[a]	1.23	12.75	39.79	56.39	56.50
8d[a]	2.22	18.75	43.32	63.65	91.25
9d[a]	1.72	9.75	38.43	63.50	78.50
10d[a]	0.74	11.75	37.53	61.65	79.25

[a] 10 mg kg^{-1} body mass.

The methyl and ethyl substituents on S at position 2 in compounds **4d** and **5d** most probably undergo hydroxylation to primary and secondary alcohols respectively (31, 32). In the case of *n*-propyl (compound **7d**) and isopropyl (compound **8d**) substituents, they are hydroxylated at the ω-1 carbon atom (31, 32) to produce secondary and tertiary alcohols, respectively. The allyl substituent (compound **6d**) undergoes hydroxylation at the allylic carbon atom (31) to produce a secondary alcohol. The initial alcohol metabolites formed from these enzymatic ω, ω-1 and allylic oxidations are susceptible to further oxidations to yield aldehydes (then acids) or ketones. Alternatively, the alcohol metabolites may undergo glucuronide conjugation. The rate of the latter reaction is faster with primary than secondary and tertiary alcohols due to steric factors. Taking these facts into account, it is evident that the rates of metabolism of the C3-containing compounds **6d**, **7d**, and **8d** will be delayed compared to those of compounds **4d** and **5d**.

On the other hand, the paw edema test revealed a marked decrease in anti-inflammatory activity upon replacement of the *p*-chlorobenzoyl moiety by H (**3e**) or benzoyl (**8c**) substituents at N1. These results comply with the previously reported findings of Black *et al.* (29) and *Kalgutkar et al.* (30).

Results for test compounds **3e**, **4d**, **5d**, **6d**, **7d**, **8c**, **8d**, **9d**, and **10d** (Table V) after 3 h revealed that the analgesic activity of all test compounds (except **3e** and **8c**) supersedes that of indomethacin and this once again explains the importance of the *p*-chlorobenzoyl substituent at N1. However, only compounds **6d**, **7d**, and **8d** were more analgesic than indomethacin after 5 h, which may be attributed to the different substituents at the S atom in position 2 which might affect their metabolic pathways, and hence the different analgesica was elicited as mentioned before (31, 32).

Table V. Analgesic activity of test compounds in the hot-plate test in mice

Compound	Reaction time (s)[b]				
	Time after injection of test compound (h)				
	0.5	1	2	3	5
5% gum acacia	8.6 ± 0.74	8.1 ± 0.74	9.0 ± 1.0	8.0 ± 1.3	7.0 ± 0.2
Indomethacin[a]	10.6 ± 0.8[d]	14.6 ± 0.8[c]	20.7 ± 2.2[d]	25.8 ± 1.1[d]	29.2 ± 0.5[d]
3e[a]	9.8 ± 0.2[d]	13.8 ± 1.3[d]	18.6 ± 2.2[d]	21.4 ± 1.9[d]	22.2 ± 0.9[d]
4d[a]	11.8 ± 0.8[d]	17.8 ± 1.3[d]	21.6 ± 2.2[d]	26.6 ± 1.9[d]	22.2 ± 0.9[d]
5d[a]	12.3 ± 0.8[d]	18.5 ± 0.7[d]	24.1 ± 1.1[d]	28.2 ± 1.4[d]	24.2 ± 1.5[d]
6d[a]	14.3 ± 0.9[d]	21.0 ± 1.3[d]	26.3 ± 1.7[d]	32.3 ± 1.7[d]	38.1 ± 0.7[d]
7d[a]	15.2 ± 1.8[d]	22.2 ± 0.1[d]	34.5 ± 0.9[d]	34.9 ± 0.9[d]	42.5 ± 0.4[c]
8c[a]	10.2 ± 0.8[d]	14.5 ± 0.7[d]	19.1 ± 1.1[d]	22.2 ± 1.4[d]	23.2 ± 0.5[d]
8d[a]	15.5 ± 1.1[d]	23.1 ± 0.2[d]	36.8 ± 2.5[d]	45.3 ± 1.4[d]	48.6 ± 1.0[d]
9d[a]	11.2 ± 0.7	15.1 ± 0.7	19.0 ± 1.1	28.0 ± 1.3	17.0 ± 0.2
10d[a]	14.2 ± 1.8[d]	18.2 ± 0.1[d]	24.5 ± 0.9[d]	25.9 ± 0.9[d]	11.5 ± 0.4[c]

[a] 10 mg kg^{-1} body mass.
[b] Values are the mean ± SEM of five observations.
Significant difference *vs.* control value (Student *t*-test): [c] $p < 0.05$, [d] $p < 0.01$.

Ulcerogenic effects

Compound **8d** that exhibited a potent analgesic and anti-inflammatory profile in the pre-mentioned animal model was evaluated for its ulcerogenic effect on rats (23). The compound showed a superior GI safety profile (17 and 50%) at oral doses 10 and 30 mg kg^{-1} respectively, when compared to indomethacin which was found to cause 67 and 100% ulceration, respectively (Table VI).

*Table VI. Ulcerogenic effects of compound **8d** in comparison with indomethacin*

Compound	Dose (mg kg^{-1})	Ratio of ulcerated animals	Ulcer index (mean ± S.E.)
	10	4/6	1.8 ± 0.1
Indomethacin	30	6/6	2.1 ± 0.2
	50	Not tested	–
	10	1/6	1.10
8d	30	3/6	1.2 ± 0.1
	50	5/6	1.3 ± 0.1

Acute toxicity

The median lethal dose (LD_{50}) of the most active compound **8d** was determined (*i.p.*) in mice and was found to be 275 mg kg^{-1}.

CONCLUSIONS

A number of 1-acyl-2-alkylthio-1,2,4-triazolo[3,2-a]benzimidazole derivatives were synthesized and tested for their antifungal activities. Some of the tested compounds showed activities comparable to that of fluconazole. Carrageenan induced paw edema and hot-plate tests revealed potent anti-inflammatory and analgesic activities for seven tested compounds. These compounds incorporate a *p*-chlorobenzoyl substituent at N1, while compounds **3e** (H) and **8c** (benzoyl) elicited less potent anti-inflammatory and analgesic activities. These results are in agreement with the reported data. In addition, the most active compound **8d** showed a superior GI safety profile compared to indomethacin.

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S A Ž E T A K

Sinteza 1-acil-2-alkiltio-1,2,4-triazolobenzimidazola s antimikotskim, protuupalnim i analgetskim učinkom

BAHAA G. MOHAMED, ABDEL-ALIM M. ABDEL-ALIM i MOSTAFA A. HUSSEIN

Sintetizirani su novi derivati 1,2,4-triazolo[2,3-a]benzimidazola reakcijom 1,2-diaminobenzimidazola s ugljičnim disulfidom. Nastali 1,2,4-triazolo[2,3-a]benzimidazol-2-tioni reagiraju s jednim ekvivalentom odgovarajućeg alkil halida pri čemu nastaju 2-alkiltio

derivati **3a-g**. Acilacijom produkata **3** dobiveni su derivati 1-acil-2-alkiltio-1,2,4-triazolo[2,3-a]-benzimidazola **4-10** u dobrim iskorištenjima. Strukture novih spojeva potvrđene su spektroskopskim metodama i elementarnom analizom. Ispitivano je antimikotsko djelovanje 14 pripravljenih spojeva, uz flukonazol kao poredbenu tvar. Većina testiranih spojeva djeluje na *Candida albicans* i *Fusarium oxysporum* slično kao i flukonazol. Najaktivniji su bili spojevi **8a**, **9a** i **10d**. Produkti **3e**, 4**c**, **5c**, **6c**, **7c**, **8b**, **8c**, **9c** i **10c** ispitani su na protuupalno i analgetsko djelovanje, uz indometacin kao poredbenu tvar. Većina spojeva ima značajno djelovanje. Najaktivniji spoj **8d** testiran je na ulcerogenost. Srednja letalna doza (LD_{50}) određena je na miševima i iznosi 275 mg kg^{-1} (*i.p.*).

Ključne riječi: 1,2-diaminobenzimidazol, hidroksilamin-*O*-sulfonska kiselina, 1,2,4-triazolo-[2,3-a]benzimidazol, antimikotsko djelovanje, analgetsko djelovanje, protuupalno djelovanje

Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University
Assiut-71526, Egypt