Bull. Pharm. Sci., Assiut University, Vol. 29, Part 2, December 2006, pp. 348-370.

SYNTHESIS AND BIOLOGICAL ACTIVITY OF CERTAIN NEW INDOLE DERIVATIVES VIA THE UTILITY OF 2-ACETYLINDOLE

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يختص البحث بتخليق بعض مشتقات١ مستبدل-إندول ٣،٢ دايون ٢ ثيوسيميكربازون الجديدة واختبارها كمضادات للميكروبات والالتهاب ، مسكنات للآلام ومضادات للتشنجات فقد تم تحضير مركبات ١ مستبدل اندول ٣،٢ دايون ٢ ثيوسيميكربازون المطلوبة (**2a, 3a, 4a**) بالتكاثف المباشر لمركب ٢ أستيل ٣ هيدروكسي ١ مستبدل إندول المناسب مع الثيوسيميكربازيد. أما تحضير مركبات ١ مستبدل-اندول ٣،٢ دايون ٢ ثيوسيميكربازون (**2b-h, 3c,e-h, 4b-h**) فقد تم من خلال تفاعل ٢ أستيل ٣ هيدروكسي ١ مستبدل إندول مع ٤ مستبدل ٣ ثيوسيميكربازيد. وقد تم التحقق من درجة نقاوة كافة المركبات الجديدة المحضرة فى هذا العمل بواسطة كروماتوجرافيا الطبقة الرقيقة كما تم التأكد من صحة التراكيب البنائية لهذه المركبات بواسطة التحاليل الدقية للعناصر والقياسات الطيفية مثل الأشعة تحت الحمراء ، الرنين المغناطيسى وكذلك مطياف الكتلة. وقد تم اختبار كل المركبات الجديدة كمضادات للبكتيريا بالمقارنة بعقار الكلورامفينيكول ومضادات للفطريات بالمقارنة بعقار الفلوكونازول كأدوية مرجعية. كما تم اختبار هذه المركبات الجديدة كمضادات للإلتهاب وقد أثبتت النتائج أن الاستبدال على نوية الإندول بمجموعة البنزيل على الموضع ١ هو محدد ضرورى في هذه المركبات لتأثيرها كمضادات للإلتهابات. كذلك أوضح اختبار الفاعلية للمركبات (**3g, 4a, 4b, 4e, 4h**) كمسكنات للآلام أن هذه المركبات اعطت نتيجتها بصورة اسرع واكثر فاعلية من عقار الإندوميثازين. هذا وقد تم اختبار تأثير المركبات (**4a, 4b, 4h**) كمسببات للقرحة المعدية كتأثير جانبي وقد أوضحت النتائج أن مركبى **4b** و **4h** ليس لهم أى نشاط مسبب للقرحة عند الجرعات العلاجية. كما أوضح اختبار السمية الحادة أن مركبي **4b** و **4h** اقل سمية مقارنة بعقار الإندوميثازين كدواء مرجعي. وعند اختبار الفاعلية المضادة للتشنجات لتلك المركبات الأربع والعشرين عند جرعة ١,٤ مللي مول/كجم اظهرت هذه المركبات نتائج مقبولة مقارنة بعقار الديازيبام كعقار مرجعى ضد التشنجات المحدثة كيميائياً. بالإضافة إلى ذلك

Received in 20/6/2006 & Accepted in 3/8/2006

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فإن المركبات السابق ذكرها اثبتت خلوها من اى تاثير كمثبطات للجهاز الحركى وباسطات للعضلات الإرادية حيث حافظت الفئران على بقائها على جهاز قياس حفظ الاتزان مقارنة بالعقار المرجعى الذى عمل على تقليل فترة بقاء الفئران على الجهاز.

*The present work involves synthesis of 1-substitutedindole-2,3-dione-2-thiosemicarbazone derivatives in order to evaluate their antimicrobial, anti-inflammatory, analgesic, and anticonvulsant activities. The target compounds were prepared through oxidative deacetylation of 1-substituted-2-acetyl-3-hydroxyindole, which followed by the condensation with different thiosemicarbazide derivatives. The purity of all the newly synthesized compounds was checked by TLC and elucidation of their structures was confirmed by IR, ^{1}H-NMR, and some representatives by mass spectrometry along with elemental microanalyses. Preliminary in-vitro antimicrobial evaluation (MIC) against some gram-positive and gram-negative bacteria as well as some fungi revealed that the tested compounds showed variable degrees of antibacterial activity, with little antifungal activity in comparison to chloramphenicol and fluconazole as reference drugs respectively. In addition, some of the tested compounds showed anti-inflammatory activity comparable to that of indomethacin. The most active compounds were further evaluated for their analgesic activity; results showed that these compounds were more active than indomethacin. Three compounds were tested for their ulcerogenicity; two of them were safer than indomethacin. Furthermore, in-vivo anticonvulsant evaluation of the tested compounds at 2.8, 2.0 and 1.4 mmol/Kg concentrations showed comparable anticonvulsant activity to that of diazepam using pentylenetetrazole induced seizure protocol. Moreover, LD_{50} of the most active compounds **4b** and **4h** were found to be 250 and 300 mg/Kg; respectively in comparison to the reported one for indomethacin 13 mg/Kg (i.p.). Moreover, the study involved the docking of the most active compound in the active site of COX-2 enzyme.*

INTRODUCTION

Literature survey revealed that indoles have been a topic of research interest.[1] Moreover, a great interest has been focused on indomethacin;[2] [1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid], a leading drug as an anti-inflammatory. Despite of its adverse effects (gastric ulceration and haemorrhage), it has been successfully utilized for the

treatment of different inflammatory disorders.[3] A large number of 2-aroylindole derivatives was synthesized and some of them showed higher anti-inflammatory activity than indomethacin and with no ulcerogenic effect.[4-7] It was observed that the combination of two or more heterocyclic moieties enhances the biological profile many folds than the parent indole nucleus.[8] In addition, certain isatin (indole-2,3-dione) derivatives were prepared and showed anti-inflammatory activity.[9] On the other hand, isatin derivatives were reported to possess a wide range of biological activities such as antibacterial,[10] antifungal,[11] antitubercular,[12] antiviral,[13] cytotoxic,[14] and anticonvulsant[15] activities.

It is interesting to mention that, nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin synthesis via the cyclooxygenase (COX) enzyme, the key to both therapeutic benefits and toxicity. COX-2 inhibitors are the second generation of NSAIDs that selectively block the COX-2 isoenzyme without affecting COX-1 function leading to control of pain and inflammation with a lower rate of adverse effects compared with the first generation.[16]

Enlightened by the aforementioned information and findings, we have undertaken the present work motivated with two objectives. First, we have been interested in the indole field from the stand point of biological activity and it was hoped that the thiosemicarbazide condensation at position 2 of indole would afford versatile derivatives in this respect. Second, we were interested in studying the results of the thiosemicarbazide condensation in a case where one could vary the substitution on the terminal nitrogen of thiosemicarbazide moiety and also on the indolic nitrogen and testing their antibacterial, antifungal, anti-inflammatory, analgesic and anti-convulsant activities, if any.

EXPERIMENTAL

Chemistry

All melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, SMP1, and UK) and were uncorrected. Precoated silica gel plates (kieselgel 0.25 mm, 60G F254, Merck) were used for monitoring of the reactions. Visualization was effected by ultraviolet lamp (model CM-10, USA) and / or iodine stain. Silica gel (60-120 mesh, Prolabo) was used for column chromatography using hexane/ethyl acetate as a mobile phase unless otherwise stated. IR spectra (KBr discs) were recorded on a Shimadzu 200-91527 spectrophotometer at the Faculty of Pharmacy, Assiut University. ^{1}H-NMR spectra were scanned on a Varian EM-360 L NMR spectrophotometer (60 MHz) at the Faculty of Pharmacy, Assiut University. Chemical shifts were expressed in δ-values (ppm) relative to TMS as an internal standard, using $CDCl_3$ and DMSO-d_6 as solvents unless otherwise stated, and deuterium oxide

was used for the detection of the exchangeable protons. Mass spectra were carried out on a JEOL JMS600 mass spectrometer at the Microanalytical Centre, Faculty of Science, Cairo University. Elemental analyses were performed on a Perkin Elmer 240 elemental analyzer at the central laboratory, Faculty of Science, Assiut University. The docking of compound 4h in the active site of COX-2 enzyme was carried out at the Pharm. Med. Chemistry department, Faculty of Pharmacy, Assiut University.

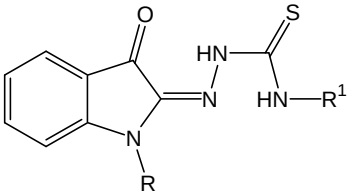
The starting materials used for the synthesis of the different intermediates required in this work are commercially available from Aldrich and include chloroacetone, anthranilic acid, alkyl or aralkyl halides and certain alkyl or aryl isothiocyanates. All other chemicals and solvents are of the reagent grade. *N*-substituted anthranilic acids,[17] 2-acetyl-3-hydroxy-1-substitutedindole (compounds **1a-c**),[17] 4-(un)substituted phenyl isothiocyanate,[18] and 4-substituted-3-thiosemicarbazide,[19] were prepared according to reported procedures.

General procedure for synthesis of 1-substituted indol-2,3-dione-2-thiosemicarbazones (compounds 2a, 3a, and 4a)

To a solution of 2-acetyl-3-hydroxy-1-substitutedindole **1a-c** (3.40 mmol) in 20 mL of ethanol, 16 mL of water, and 8 mL of concentrated hydrochloric acid were added thiosemicarbazide (0.5 g, 5.5 mmol). The mixture was refluxed for 12 h (as indicated by TLC; hexane: ethyl acetate 6:4) then cooled and filtered. The yellow hydrochloride salts were dissolved in hot water and filtered. To the hot filtrate was added 10 mL of 5% sodium bicarbonate solution and the mixture was stirred for 1 h. The yellow precipitates that formed were filtered, washed with water and recrystallized from ethanol. Physical, microanalytical and spectral data are given in Tables 1 and 2.

Meanwhile, the thiosemicarbazones (compounds **2b-h**, **3c**,**e-h** and **4b-h**) were prepared by stirring a solution of the appropriate 2-acetyl-3-hydroxy-1-substitutedindole (5.0 mmol) in 10 mL of ethanol, the appropriate substituted thiosemicarbazide (5.0 mmol) was added. The reaction mixture was further stirred at the ambient temperature for 3-10 days (monitored by TLC; hexane / ethyl acetate 6:4) then it was concentrated under vacuum and the solid products obtained by filtration were recrystallized from aqueous ethanol and dried. Physical, microanalytical and spectral data are given in Tables 1 and 2.

Table 1: Physical and microanalytical data of compounds **2-4**.

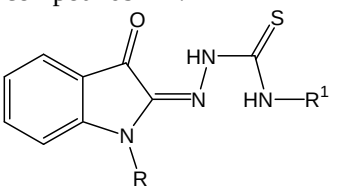


No.	R	R^1	Yield %	Reac. Time (day)	M.p.°	M.F/ M.Wt	Microanalysis Calculated/Found			
							C%	H%	N%	S%
2a	CH_3	H	30	0.5	238-40	$C_{10}H_{10}N_4OS$ (234.28)	51.27 51.47	4.30 4.03	--	13.69 13.10
2b	CH_3	CH_3	25	7	200-02	$C_{11}H_{12}N_4OS$ $^1/_2H_2O$ (257.30)	51.35 51.63	5.09 4.28	21.77 21.97	12.46 12.76
2c	CH_3	C_2H_5	30	5	198-99	$C_{12}H_{14}N_4OS$ $^1/_2$ H_2O (271.32)	53.12 53.97	5.57 5.35	20.64 20.90	11.81 11.66
2d	CH_3	$CH_2CH=CH_2$	30	3	164-66	$C_{13}H_{14}N_4OS$ $^1/_2H_2O$ (283.33)	55.10 55.34	5.33 4.87	19.77 19.36	--
2e	CH_3	*c*-C_6H_{11}	27	3	212-14	$C_{16}H_{21}N_4OS$ $^1/_2H_2O$ (325.42)	59.05 59.97	6.50 6.15	17.20 17.50	9.85 9.05
2f	CH_3	C_6H_5	24	5	178-80	$C_{16}H_{14}N_4OS$ (310.37)	61.92 61.30	4.55 4.44	18.05 17.87	--
2g	CH_3	*p*-CH_3-C_6H_4	53	5	182-84	$C_{17}H_{16}N_4OS$ $^1/_2H_2O$ (333.41)	61.24 60.82	5.13 4.54	16.80 16.66	--
2h	CH_3	*p*-Cl-C_6H_4	34	8	186-88	$C_{16}H_{13}ClN_4OS$ (344.81)	55.73 55.30	3.80 3.28	16.25 16.14	9.30 8.71
3a	C_2H_5	H	40	0.5	192-94 (dec.)	$C_{11}H_{12}N_4OS$ (248.30)	53.21 52.66	4.87 4.63	--	--
3c	C_2H_5	C_2H_5	35	7	122-24	$C_{13}H_{16}N_4OS$ (276.35)	56.50 56.43	5.84 5.27	20.27 20.43	11.60 11.59
3e	C_2H_5	*c*-C_6H_{11}	27	3	127-29	$C_{17}H_{22}N_4OS$ (330.447)	61.79 61.29	6.71 6.48	16.95 16.72	9.70 9.22
3f	C_2H_5	C_6H_5	70	5	186-88	$C_{17}H_{16}N_4OS$ (324.40)	62.94 62.70	4.97 4.95	17.27 17.26	9.88 9.02
3g	C_2H_5	*p*-CH_3-C_6H_4	63	7	184-86	$C_{18}H_{18}N_4OS$ (338.42)	63.88 63.76	5.36 5.16	16.56 16.57	--
3h	C_2H_5	*p*-Cl-C_6H_4	31	10	196-98	$C_{17}H_{15}ClN_4OS$ (358.85)	56.90 56.10	4.21 4.23	15.61 15.46	--
4a	Bz	H	57	0.5	248-50	$C_{16}H_{14}N_4OS$ (310.37)	61.92 61.48	4.55 4.51	18.05 17.66	10.33 10.55

Table 1: Continued.

No.	R	R^1	Yield %	Reac. Time (day)	M.p.°	M.F/ M.Wt	Microanalysis Calculated/Found			
							C%	H%	N%	S%
4b	Bz	CH_3	60	3	218-20	$C_{17}H_{16}N_4OS$ $^1/_2H_2O$ (333.40)	61.24 61.20	5.13 4.52	16.80 16.80	9.61 8.93
4c	Bz	C_2H_5	57	3	198-200	$C_{18}H_{18}N_4OS$ $1H_2O$ (356.44)	60.65 61.14	5.65 5.04	15.71 15.53	8.99 9.55
4d	Bz	$CH_2CH=CH_2$	84	3	130-32	$C_{19}H_{18}N_4OS$ $^1/_2$ H_2O (359.44)	63.49 63.17	5.32 4.79	15.58 15.01	--
4e	Bz	*c*-C_6H_{11}	61	3	172-74	$C_{22}H_{24}N_4OS$ (392.51)	67.32 66.83	6.16 6.17	14.27 14.16	8.17 7.67
4f	Bz	C_6H_5	40	5	174-76	$C_{22}H_{18}N_4OS$ $^1/_4$ H_2O (390.97)	67.52 67.39	4.60 4.64	14.32 14.40	8.20 8.50
4g	Bz	*p*-CH_3-C_6H_4	53	5	182-84	$C_{23}H_{20}N_4OS$ (400.49)	68.98 68.52	5.03 4.90	13.99 13.94	8.01 8.17
4h	Bz	*p*-Cl-C_6H_4	45	10	245-47	$C_{22}H_{17}ClN_4OS$ $1H_2O$ (438.92)	60.20 59.66	4.36 3.74	12.76 13.05	--

Table 2: ^{1}H-NMR data of compounds **2-4**.



No.	R	R^1	^{1}H-NMR ($CDCl_3$, δ ppm)*
2a**	CH_3	H	3.40 (s, 3H, NCH_3); 7.40-8.60 (m, 4H, $C_6\underline{H}_4$); 9.20 and 9.80 (br.s, $N^4\underline{H}_2$); 13.21 (br.s, 1H, $N^2\underline{H}$).
2b	CH_3	CH_3	3.27 (d, 3H, $N^4HC\underline{H}_3$); 3.47 (s, 3H, NCH_3); 6.90-7.60 (m, 4H, $C_6\underline{H}_4$); 8.80 (br.s, 1H, $N^4\underline{H}$); 12.05 (s, 1H, $N^2\underline{H}$).
2c	CH_3	C_2H_5	1.31 (t, 3H, $CH_2C\underline{H}_3$); 3.40 (s, 3H, NCH_3); 3.51-4.21 (m, 2H, $C\underline{H}_2CH_3$); 7.00-8.11 (m, 5H, $C_6\underline{H}_4$ and $N^4\underline{H}$); 12.81 (s, 1H, $N^2\underline{H}$).
2d	CH_3	$CH_2CH=CH_2$	3.50 (s, 3H, NCH_3); 4.61 (t, 2H, $NC\underline{H}_2CH=CH_2$); 5.50 (d, 1H, $=C\underline{H}_2$); 5.71 (dd, 1H, $=C\underline{H}_2$); 6.00-6.80 (m, 1H, $CH_2C\underline{H}=CH_2$); 7.11-8.50 (m, 4H, $C_6\underline{H}_4$); 8.90 (br.t, 1H, $N^4\underline{H}$); 13.40 (s, 1H, $N^2\underline{H}$).
2e	CH_3	*c*-C_6H_{11}	0.70-2.70 (m, 10H, *c*-$C_6\underline{H}_{11}$); 3.50 (s, 3H, NCH_3); 4.20-5.00 (m, 1H, *c*-$C_6\underline{H}_{11}$); 7.00-8.50 (m, 5H, $C_6\underline{H}_4$ and $N^4\underline{H}$); 13.10 (br.s, 1H, $N^2\underline{H}$).
2f	CH_3	C_6H_5	3.51 (s, 3H, NCH_3); 7.20-8.60 (m, 9H, $C_6\underline{H}_4$ and $C_6\underline{H}_5$); 9.90 (br.s, 1H, $N^4\underline{H}$); 13.40 (br.s, 1H, $N^2\underline{H}$).

Table 2: Continued.

No.	R	R^1	^{1}H-NMR ($CDCl_3$, δ ppm)*
2g**	CH_3	*p*-CH_3-C_6H_4	2.50 (s, 3H, *p*-C<u>H</u>$_3$-C_6<u>H</u>$_4$); 3.60 (s, 3H, NC<u>H</u>$_3$); 7.21-8.41 (m, 8H, C_6<u>H</u>$_4$, and *p*-CH_3-C_6<u>H</u>$_4$); 10.30 (s, 1H, N^4<u>H</u>); 12.70 (s, 1H, N^2<u>H</u>).
2h	CH_3	*p*-Cl-C_6H_4	3.61 (s, 3H, NC<u>H</u>$_3$); 7.20-8.5(m, 8H, C_6<u>H</u>$_4$ and *p*-Cl-C_6<u>H</u>$_4$); 10.80 (br.s, 1H, N^4<u>H</u>); 13.10 (br.s, 1H, N^2<u>H</u>).
3a**	C_2H_5	H	1.10 (t, 3H, NCH$_2$C<u>H</u>$_3$); 3.90, 4.36 (2q, 2H, NC<u>H</u>$_2$CH$_3$); [50%, 50%]; 7.20-8.45 (m, 4H, C_6<u>H</u>$_4$); 8.73, 9.10, 9.40, 9.60 (br.s, N^4<u>H</u>$_2$); 13.30 (s, 1H, N^2<u>H</u>).
3c	C_2H_5	C_2H_5	1.40 (t, 6H, N^4CH$_2$C<u>H</u>$_3$ and NCH$_2$C<u>H</u>$_3$); 3.61-4.40 (m, 4H, Ind NC<u>H</u>$_2$CH$_3$ and N^4C<u>H</u>$_2$CH$_3$); 7.10-8.50 (m, 5H, C_6<u>H</u>$_4$ and N^4<u>H</u>); 13.11 (s, 1H, N^2<u>H</u>).
3e	C_2H_5	*c*-C_6H_{11}	0.80-2.50 (m, 13H, 10H of *c*-C_6<u>H</u>$_{11}$and CH$_2$C<u>H</u>$_3$); 3.90 (q, 2H, C<u>H</u>$_2$CH$_3$); 4.10-4.70 (m, 1H, *c*-C_6<u>H</u>$_{11}$); 6.70-7.90 (m, 5H, C_6<u>H</u>$_4$ and N^4<u>H</u>); 12.50 (s, 1H, N^2<u>H</u>).
3f	C_2H_5	C_6H_5	1.30 (t, 3H, CH$_2$C<u>H</u>$_3$); 4.33 (q, 2H, C<u>H</u>$_2$CH$_3$); 7.30-8.46 (m, 9H, C_6<u>H</u>$_4$ and C_6<u>H</u>$_5$); 11.16 (s, 1H, N^4<u>H</u>); 13.26 (br.s, 1H, N^2<u>H</u>).
3g	C_2H_5	*p*-CH_3-C_6H_4	1.40 (t, 3H, CH$_2$C<u>H</u>$_3$); 2.51 (s, 3H, *p*-C<u>H</u>$_3$-C_6H$_4$); 4.11 (q, 2H, C<u>H</u>$_2$CH$_3$); 7.30-8.60 (m, 8H, C_6<u>H</u>$_4$ and *p*-CH_3-C_6<u>H</u>$_4$); 9.81 (s, 1H, N^4<u>H</u>); 13.50 (s, 1H, N^2<u>H</u>).
3h**	C_2H_5	*p*-Cl-C_6H_4	1.30 (t, 3H, NCH$_2$C<u>H</u>$_3$); 4.30 (q, 2H, Ind NC<u>H</u>$_2$CH$_3$); 7.25-8.40 (m, 8H, C_6<u>H</u>$_4$ and *p*-Cl-C_6<u>H</u>$_4$); 11.06 (br.s, 1H, N^4<u>H</u>); 13.16 (s, 1H, N^2<u>H</u>).
4a**	Bz	H	5.40 (s, 2H, C_6H_5C<u>H</u>$_2$); 7.30-8.50 (m, 9H, C_6<u>H</u>$_4$ and $C_6H_5CH_2$); 9.20 and 9.70 (br.s, 2H, N^4<u>H</u>$_2$); 13.41 (s, 1H, N^2<u>H</u>).
4b**	Bz	CH_3	3.2 (d, 3H, N^4C<u>H</u>$_3$); 5.60 (s, 2H, C_6H_5C<u>H</u>$_2$); 7.20-8.40(m, 9H, C_6<u>H</u>$_4$, and C_6<u>H</u>$_5$CH$_2$); 9.40-9.60 (br.q, 1H, N^4H); 13.20 (s, 1H, N^2H).
4c***	Bz	C_2H_5	2.20 (t, 3H, CH$_2$C<u>H</u>$_3$); 3.60-4.30 (m, 2H, C<u>H</u>$_2$CH$_3$); 5.50 (s, 2H, C_6H_5C<u>H</u>$_2$); 7.30-8.40 (m, 9H, C_6<u>H</u>$_4$, and CH$_2$$C_6$<u>H</u>$_5$); 8.90-9.00(br.t, 1H, N^4<u>H</u>); 13.21(s, 1H, N^2<u>H</u>).
4d**	Bz	$CH_2CH=CH_2$	4.50 (t, 2H, NC<u>H</u>$_2$CH=CH$_2$); 5.31-5.50 (m, 2H, =C<u>H</u>$_2$); 5.51 (s, 2H, C_6H_5C<u>H</u>$_2$); 5.90-6.60 (m, 1H, C<u>H</u>); 7.30-8.40 (m, 9H, C_6<u>H</u>$_5$, and C_6<u>H</u>$_5$CH$_2$); 9.70 (br.t, 1H, N^4<u>H</u>); 14.10 (s, 1H, N^2<u>H</u>).
4e	Bz	*c*-C_6H_{11}	0.50-2.60 (m, 10H, *c*-C_6<u>H</u>$_{11}$); 4.00-4.70 (m, 1H, *c*-C_6<u>H</u>$_{11}$); 5.00 (s, 2H, C_6H_5C<u>H</u>$_2$); 6.50-7.90 (m, 9H, C_6<u>H</u>$_4$, and $C_6H_5CH_2$); 8.06 (br.s, 1H, N^4<u>H</u>); 12.40 (s, 1H, N^2<u>H</u>).
4f**	Bz	C_6H_5	5.20 (s, 2H, C_6H_5C<u>H</u>$_2$); 6.70-8.30 (m, 14H, C_6<u>H</u>$_4$, $C_6H_5CH_2$, and C_6<u>H</u>$_5$); 9.90 (br.s, 1H, N^4<u>H</u>); 12.60 (br.s, 1H, N^2<u>H</u>).
4g	Bz	*p*-CH_3-C_6H_4	2.20 (s, 3H, *p*-C<u>H</u>$_3$-C_6H$_4$); 5.10 (s, 2H, C_6H_5C<u>H</u>$_2$); 6.80-8.20 (m, 13H, C_6<u>H</u>$_4$, *p*-CH_3-C_6<u>H</u>$_4$, and C_6<u>H</u>$_5$CH$_2$); 9.20 (br.s, 1H, N^4<u>H</u>); 12.70 (br.s, 1H, N^2<u>H</u>).
4h**	Bz	*p*-Cl-C_6H_4	4.70 (s, 2H, C_6H_5C<u>H</u>$_2$); 6.50-8.20 (m, 13H, C_6<u>H</u>$_4$, $C_6H_5CH_2$, and *p*-Cl-C_6<u>H</u>$_4$); 8.90 (br.s, 1H, N^4<u>H</u>); 12.90 (s, 1H, N^2<u>H</u>).

*Proton of NH is exchangeable with D_2O, **DMSO-d_6, ***Acetone-d_6

Biological screening

A- Antimicrobrial activity

Antimicrobrial screening was determined in the Assiut University Mycological Center (AUMC). Bacterial and fungal cultures were obtained from (AUMC), Assiut university, Egypt.

Antibacterial activity

Organisms and culture conditions

Six bacterial species represent both gram-positive and gram-negative strains were used to test the antibacterial activities of the target compounds: *Bacillus cereus*, *Micrococcus luteus*, and *Staphylococcus aureus* as representatives for the gram-positive strains, while the gram-negative strains were represented by *Escherichia coli*, *Serratia marcescens*, and *Pseudomonas aeroginosa*.

Materials and methods[20]

Cell suspension of bacterial strains was prepared from 48 h old cultures, grown on Nutrient Agar (NA) in sterilized water. One mL suspension was added to tubes containing 15 mL of NA. The tubes were shaken gently to homogenize the inoculums. The cultures were then diluted until they equaled to the turbidity of the standard. The bacterial suspensions were added to solutions of serial dilutions of the tested compounds **2-4** and chloramphenicol in DMSO. The seeded tubes were incubated at 35 ± 2° for 24-48 h. The minimal inhibitory concentrations (MIC) values were read as the greatest dilution (least concentration) that completely inhibited any visible bacterial growth and the results are given in Table 3.

Antifungal activity

Organisms and culture conditions

Eight pathogenic, phytopathogenic and food poisoning fungal species were used in the present study: *Trichophyton rubrum* (404), *Candida albicans* (3374), *Asperigillus flavus* (40), *A. niger* (3364), *Fusarium oxysporum* (208), *Geotrichum candidum* (227), *Scopulariopsis brevicaulis* (3383), and *Penicillium expansum* (525).

Materials and method[20]

Antifungal activity of the tested compounds **2-4** and fluconazole was performed by a reported method as follow:[20]

Spore suspension in sterile distilled water was prepared from 5 days old culture of the tested fungi growing on Potato Dextrose Agar (PDA) or Sabouraud Agar (SA) media. The final spore concentration was 5×10^5 spores/mL. One mL of the spore suspension was added introduced in sterilized tubes containing about 10 mL of growth medium. The tubes were shaken gently to homogenize the inoculums.

The fungal suspensions were added to solutions of serial dilutions of the tested compounds **2-4** and fluconazole in DMSO. The seeded tubes were incubated at 35 ± 2° for 7 days. The minimal inhibitory concentration (MIC) values were read

as the greatest dilution (least concentration) that completely inhibited any visible fungal growth.

B- Pharmacological Screening

Pharmacological screening was done at Dept. of Pharmacology, Faculty of Medicine, Assiut University. Male adult albino mice and rats were obtained from the animal house, Faculty of Medicine, Assiut University. Indomethacin (Indacin® vial, Memphis Co. Egypt), carrageenan (Sigma, USA). Diazepam (Valinil® 5 mg Tablets, Nile Pharm. Co., Egypt), pentylenetetrazole (Sigma, USA), other chemicals and solvents were obtained from local market.

Anti-inflammatory activity

The anti-inflammatory activity of the newly synthesized compounds **2-4** was determined according to paw induced edema method[21] in comparison to indomethacin as a reference drug. The test is based on the pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 mL of 1% w/v solution in normal saline) into the right hind of the rats.

Method

Male adult albino rats (120-150 g) were divided into twenty four groups, each of four animals. The thickness of rat paw was measured by a Vernier caliper (SMIEC, China) before and after 1 h of carrageenan injection to detect the inflammation induced by carra-geenan. Solutions or suspensions of the tested compounds and the reference drug at a dose of 0.02 mmol/Kg in 1% w/v sodium carboxymethylcellulose (NaCMC) were injected *i.p.* to twenty three different groups of rats. Control group received a vehicle (1% NaCMC solution), while reference group received indomethacin *i.p.* at 0.02 mmol/Kg.

The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 h after injection of the tested compounds, reference drug, and the vehicle. Results of anti-inflammatory activity of the tested compounds and the reference drug are listed in Table 4. The percentage of edema inhibitions were calculated[21] where:

%Edema inhibition= $(V_R–V_L)$control–$(V_R–V_L)$treated× 100 / $(V_R–V_L)$ control

V_R: Average right paw thickness,
V_L: Average left paw thickness.

Table 3: MIC values (µmol/mL) of compounds **2-4** and chloramphenicol.

Compd. No.	*B. cereus*	*M. luteus*	*S. aureus*	*E. coli*	*S. marcescens*	*P. aeroginosa*
2a	10.6	42.6	21.3	10.6	10.6	42.6
2b	10.0	10.0	10.0	10.0	10.0	10.0
2c	9.0	9.0	9.0	9.0	9.0	9.0
2d	8.6	8.6	8.6	17.3	8.6	8.6
2e	7.9	31.6	7.9	7.9	31.6	7.9
2f	--*	--	--	--	--	--
2g	7.7	7.7	7.7	15.4	7.7	--
2h	--	--	--	--	--	--
3a	--	--	--	--	--	--
3c	9.0	9.0	9.0	9.0	9.0	36.2
3e	7.6	7.6	15.0	15.0	15.0	7.6
3f	7.7	7.7	7.7	7.7	7.7	15.4
3g	7.3	7.3	7.3	7.3	7.3	7.3
3h	6.9	6.9	6.9	6.9	13.9	6.9
4a	8.0	16.0	16.0	32.0	8.0	32.0
4b	7.7	7.7	7.7	30.8	7.7	7.7
4c	7.3	7.3	7.3	7.3	7.3	7.3
4d	7.1	7.1	7.1	7.1	7.1	7.1
4e	6.4	6.4	25.5	6.4	6.4	12.7
4f	6.5	6.5	6.5	6.5	6.5	6.5
4g	6.2	6.2	6.2	12.5	6.2	12.5
4h	5.9	5.9	5.9	5.9	5.9	--
Chloram-phenicol	7.7	7.7	7.7	15.5	--	15.5

*-- Means no inhibition.

Table 4: Inhibitory effect of compounds **2-4** and indomethacin on carrageenan induced paw edema in rats.

Compd. No.	%edema inhibition						
	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Indomethacin	2.27	41.65	52.55	63.73	67.16	69.04	84.85
2a	0.25	6.82	21.06	32.70	48.21	37.66	27.87
2b	3.02	9.41	18.09	27.6	37.89	22.59	22.20
2c	0.25	36.71	83.43	48.85	38.11	28.30	18.26
2d	2.27	28.71	41.91	48.00	42.53	32.43	22.61
2e	5.54	48.24	58.51	53.88	22.11	22.59	23.24
2f	4.79	35.76	47.23	48.00	26.74	27.20	17.43
2g	2.77	42.12	58.30	53.67	48.21	17.15	17.84
2h	5.29	24.47	37.02	43.19	37.68	22.38	23.02
3a	0.00	42.35	42.55	37.74	37.47	27.82	38.80
3c	5.29	48.00	47.66	43.19	37.68	38.08	38.59
3e	0.50	42.35	42.55	37.74	37.16	27.82	28.42
3f	2.27	18.12	26.00	16.56	16.21	16.74	17.43
3g	5.29	52.71	63.62	69.39	69.26	69.46	64.52
3h	2.27	29.41	36.17	48.00	36.84	21.00	21.58
4a	0.76	53.89	64.04	69.81	74.95	75.10	75.31
4b	3.02	53.00	63.83	64.36	64.21	69.66	75.10
4c	4.03	53.41	53.00	48.43	64.00	58.58	48.96
4d	4.79	35.76	47.23	53.25	37.26	27.20	17.43
4e	3.53	52.48	68.51	53.25	53.05	63.81	64.11
4f	0.25	25.65	21.06	27.46	27.16	27.62	27.87
4g	4.79	38.12	57.02	58.10	63.58	63.81	58.51
4h	3.02	35.29	58.51	65.41	69.47	75.00	64.73

Analgesic Activity

Hot-plate test

The analgesic activity of compounds (**3g**, **4a**, **4b**, **4e**, and **4h**) was determined in mice using the hot-plate method[21] in comparison to indomethacin. Male albino mice (30-35g) were placed for testing on a hot-plate apparatus, its surface temperature was maintained at 55±0.5°. The reaction time in seconds was taken as a time period from the instant the animal reached the hot plate until the moment the animal licked its hind paw or jumped out within a plexiglass cylinder placed on the hot-plate.[21] This reaction time was taken as the end point and the increase in hot plate latency was taken as a measure of the analgesic activity.

Method

Male adult albino mice (25-30 g) were divided into seven groups; each of four animals and each animal was

used once. Solutions or suspensions of the tested compounds and the reference drug in 1% w/v NaCMC were injected *i.p.* at a dose level of 0.02 mmol/Kg into mice. Control animals were similarly treated with 1% w/v NaCMC. Testing was done after 0.5, 1.0, 2.0, 3.0, and 5.0 h of the injection. Mean licking time ± S.E. was evaluated for each group and listed in Table 5.

Ulcerogenic effect

The test was done according to a reported method.[22] Male albino rats were fasted for 24 h and the tested compounds (**4a**, **4b**, and **4h**) and reference drug were administered orally to four groups each of 6 animals. After 6 h, the animals were sacrificed, the stomachs were removed and gastric lesions on the mucosa were determined by using stereoscopic microscope. Ulcer was defined as at least one lesion that was 0.5 mm or more in length. All lesions of more than 0.1 mm in length were summed to obtain the ulcer index. The results were listed in Table 6.

Acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of compounds (**4b** and **4h**) was determined in mice by graphical Litchfield method.[23]

Materials and method

Groups of male adult albino mice, each of four animals (25-30 g), were injected *i.p.* with graded doses of the tested compound. The percentage mortality in each group of animals was determined 72 h latter to injection. Computation of LD_{50} was processed by a graphical method.[23]

CNS depressant activity

CNS depressant activity of compounds **2-4** was evaluated by testing their anticonvulsant activity[24] in addition to their effect on a rota-rod test in comparison to diazepam by reported methods.[25]

Method

Anticonvulsant activity

Blockade of pentylenetetrazole-induced convulsions in mice is a characteristic effect of some CNS depressant drugs. All compounds were investigated for their anticonvulsant activity by following the anticonvulsant drug development (ADD) program.[24] The tested compounds and the reference drug were dissolved or suspended in 1% w/v aqueous solution of NaCMC and administered *i.p.* in mice. The elapsed time before the onset of clonic convulsions, tonic convulsions, and death were recorded. Mice have considered to be survived if they lived for longer than 20 minutes after pentylenetetrazole administration. This test was performed at four different dose levels, 2.8 and 2.0 mmol/kg (compounds **2a-h**), 1.4 mmol/kg (compounds **2-4**) and 1.0 mmol/kg (compounds **4e-h**). Results are shown in Tables 7 and 8.

The rota-rod motor coordination test

Three groups of mice each of four animals were used. The ability of mice to remain on a rotating rod for

20 seconds without falling was determined at 10 minutes intervals over a period of 2 h following the *i.p.* injection of the tested compounds **2-4** and diazepam at a dose level of 2.8 mmol/Kg in NaCMC (1% w/v aqueous solution).[25]

Table 5: The mean licking time (±S.E.) in the hot-plate test of compounds (**3g, 4a, 4b, 4e**, and **4h**) and indomethacin in mice.

Compd. No.	The average reaction time (second) at different times after compound administration ± S.E.				
	0.5 h	1 h	2 h	3 h	5 h
Control	6.24±0.18	8.60±0.43	8.10±0.73	6.50±0.57	7.00±0.81
Indomethacin	10.10±0.27**	14.74±0.30**	14.74±0.30**	20.35±0.16**	13.28±0.30**
3g	20.80±0.14**	20.85±0.17**	20.95±0.75**	12.15±0.21**	9.15±0.50**
4a	11.60±0.73**	14.30±0.16**	13.30±0.30**	7.50±1.29	7.50±1.29
4b	15.10±0.27**	20.60±0.16**	16.10±0.27**	13.00±0.08**	11.50±0.45**
4e	15.20±0.29**	27.13±0.23**	25.90±0.75**	7.35±0.93	7.35±0.93
4h	18.00±0.45**	19.20±0.35**	20.40±0.23**	15.70±0.30**	13.30±0.30**

-Values are the mean ± S.E. of four observations
-* Significant difference at $P < 0.05$ vs. control value (student's-t-test)
-** Significant difference at $P < 0.01$ vs. control value (student's-t-test)

Table 6: Ulcerogenic effects of compounds (**4a**, **4b**, and **4h**) and **indomethacin**.

Compd. No.	Dose mg/kg	Ratio of ulcerated animals	Ulcer index (mean ± S.E)
Indomethacin	10	4/6	1.85±0.23
	30	6/6	3.00±0.16
	50	Not tested	--
4a	10	2/6	1.00±0.35
	30	4/6	1.40±0.58
	50	6/6	4.00±0.35
4b	10	0/6	0.00
	30	1/6	1.20±0.25
	50	3/6	1.75±0.17
4h	10	0/6	0.00
	30	1/6	1.20±0.25
	50	3/6	1.35±0.34

Table 7: Anticonvulsant activity of compounds **2a-h** and diazepam at 2.8 mmol/Kg.

Compd. No.	Time* of clonic convulsions	Time* of tonic convulsions	No. of protected animals	No. of Dead animals	% Protection
2a	-**	-**	4	none	100
2b	-	-	4	none	100
2c	-	-	4	none	100
2d	-	-	4	none	100
2e	-	-	4	none	100
2f	-	-	4	none	100
2g	-	-	4	none	100
2h	-	-	4	none	100
Diazepam	-	-	4	none	100
Control	1	3	none	4	0***

* Time in minutes. ** No clonic or tonic convulsions.

*** Animals were died after 5-10 minutes.

Table 8: Lipophilicity value (Clog P) and anticonvulsant activity of compounds **2-4** and diazepam at 1.4 mmol/Kg.

Compd. No.	Clog P*	Anticonvulsant activity**	Mortality (%)***
Diazepam	2.84	4/4	0
2a	1.35	0/4	100
2b	1.87	1/4	75
2c	1.58	0/4	100
2d	2.70	1/4	75
2e	3.41	1/4	75
2f	3.53	1/4	75
2g	4.02	0/4	100
2h	4.09	0/4	100
3a	1.68	3/4	25
3c	2.54	4/4	0
3e	3.75	4/4	0
3f	3.87	4/4	0
3g	4.36	4/4	0
3h	4.43	3/4	25
4a	2.83	4/4	0
4b	3.35	4/4	0
4c	4.24	4/4	0
4d	4.19	4/4	0
4e	4.90	2/4	50
4f	5.02	2/4	50
4g	5.50	1/4	75
4h	5.75	1/4	75

* Calculated partition coefficient.[26]

** No. of animals protected/ total no. of animals.

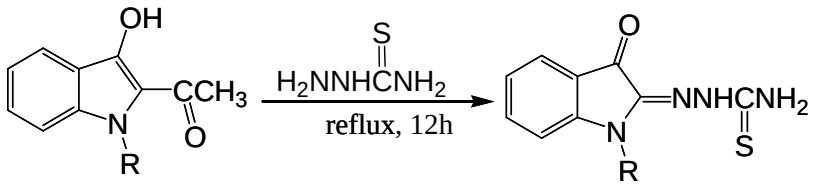
*** No. of animals died/ total no. of animals × 100.

RESULTS AND DISCUSSION

Chemistry

In the present investigation, 1-substituted-1*H*-indole-2,3-dione-2-thiosemicarbazones, compounds **2a**, **3a**, and **4a**, were prepared by refluxing 2-acetyl-3-hydroxy-1-substitutedindole.[17] (**1a-c**) with thiosemicarbazide[19] in a mixture of ethanol : water : hydrochloric acid 5 : 4 : 2 for 12 h as indicated by TLC, Scheme 1. The target compounds were obtained in yields ranged from 30-57%, and their structures were confirmed using IR, ^{1}H-NMR as well as elemental analysis (Tables 1 and 2).

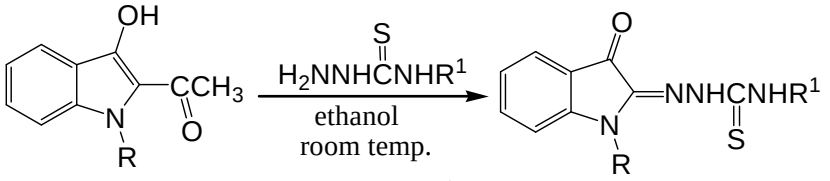
Preparation of 1-substituted-*1H*-indole-2,3-dione-2-substituted thiosemicarbazones (**2b-h**, **3c, e-h**, and **4b-h**) was carried out by stirring equimolar amounts of compounds (**1a-c**) with the appropriate substituted thiosemicarba-zide in ethanol at room temperature for 3-10 days, Scheme 2. It was noticed that these compounds could not be prepared by stirring at room temperature in acidified ethanol or refluxing in ethanol acidified either with conc. sulfuric acid, conc. hydrochloric acid or glacial acetic acid.



(**1a-c**) (**2a, 3a and 4a**)

R = CH_3, C_2H_5, $C_6H_5CH_2$

Scheme 1



R = CH_3, C_2H_5, $CH_2C_6H_5$; R^1 = CH_3, C_2H_5, CH_2CH =CH_2, *c*-C_6H_{11}, C_6H_5, 4-CH_3-C_6H_4, 4-Cl-C_6H_4.

Scheme 2

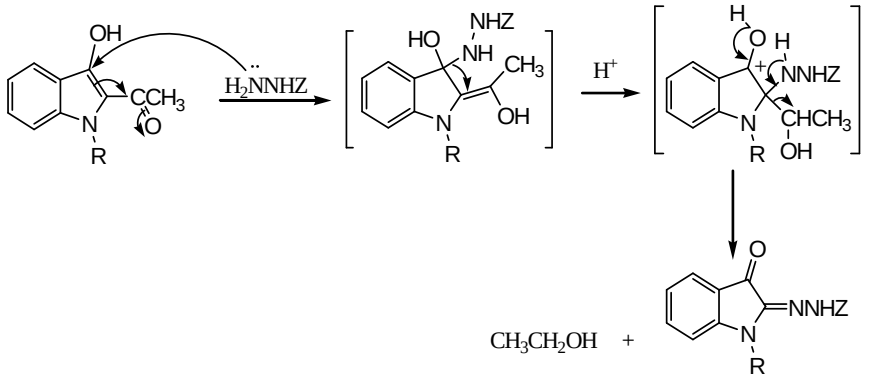
Physical and spectral data of compounds **2b-h, 3c, e-h,** and **4b-h** are given in Tables 1 and 2. Structures of the target compounds were identified by IR, ^{1}H-NMR and elemental analyses in addition to MS for representative examples. The purity of target compounds was checked by, TLC and elemental microanalyses.

IR spectra of the target thiosemicarbazones (**2-4**) lacked the bands which due to the carbonyl of acetyl group and the hydroxyl groups of the starting indoles and showed bands due to N^4H and N^2H functions at 3450-3340 cm^{-1} and 3300-3200 cm^{-1} respectively, the mixed vibrational coupling of the NCS moieties at 1560-1520 cm^{-1}, 1335-1320 cm^{-1}, 1180-1150 cm^{-1} and 950-920 cm^{-1}, as well as a band at 1590-1580 cm^{-1} characteristic for C=N and C=C functions.

It is noteworthy to mention that, the ^{1}H-NMR data of compound **2a**, Table 2**,** showed two broad singlets of different chemical shifts for the NH_2 group, which are diastereotopic protons and consequently, they are magnetically nonequivelent.[27] Similarly, the ^{1}H-NMR data of compound, **3a**, Table 2**,** revealed the presence of duplicate sets of protons as such compound was produced as *cis-trans* isomeric mixture.[27]

Mass spectrum of compound **2c**, revealed the molecular ion peak M^+ at (*m/z* 262, 54.5%) corresponding to the molecular weight of this compound and a base peak at (*m/z* 161,100%). Mass spectrum of compound **2e**, revealed the molecular ion peak M^+ at (*m/z* 316, 55.2%) in addition to a base peak at (*m/z* 161, 100%). Mass spectrum of compound **3c**, revealed the molecular ion peak M^+ at (*m/z* 276, 52.9%) corresponding to the molecular weight of this compound and a base peak at (*m/z* 146, 100%). Mass spectrum of compound **3g**, revealed the molecular ion peak M^+ at (*m/z* 338 64.7%) corresponding to the molecular weight of this compound. Also the spectrum showed prominent peaks at (*m/z* 106; intensity 100%), (*m/z* 146; intensity 78.1%) and (*m/z* 77; intensity 81.7%). MS spectrum of compound **4d**, revealed the molecular ion peak M^+ at (*m/z* 350 5.9%) and a base peak at (*m/z* 91 100%). MS spectrum of compound **4e**, showed the molecular ion peak M^+ at (*m/z* 392 10.8%), in addition to a base peak at (*m/z* 91 100%) and the characteristic fragmentation pattern of cyclohexyl moiety.

The suggested mechanism for the present reaction is based on reported data,[28] oxidative rearrangement and conversion of indoles to oxindoles in high yields in protic solvents such as ethyl alcohol where, acetyl group displaced with its electrons.[28] The mechanism of formation of de-acetylated derivatives involves nucleophilic addition to the active carbon to give an indolenine intermediate, followed by 1,2 migration with the displacement of the acetyl group to form carbon nitrogen double bond in the second step, Scheme 3**.**



Scheme 3

Biological screening

A- Antimicrobial activities

Antibacterial activity

The newly synthesized compounds **2-4** were tested for their *in vitro* antibacterial activity using a serial dilution method[20] against *Bacillus cereus, Micrococcus luteus,* and *Staphylococcus aureus* as represen-tatives of gram-positive strains and *Escherichia coli, Serratia marcescens*, and *Pseudomonas aeroginosa* as representatives of gram-negative ones, using chlora-mphenicol as a reference drug. The results of MIC are cited in Table 3.

Results of the antibacterial activity of the 1-methyl derivatives, compounds **2a-h**, Table 3, indicated that six compounds **2a-e** and **2g** showed moderate to excellent antibacterial activities. Also, it was observed that compound **2c** was the most active derivative against all species of bacteria, while compounds **2f** and **2h** were inactive against all species of bacteria. Results of the 1-ethyl derivatives, compounds **3a, c, e-h** Table 3, revealed that compound **3a** (R = C_2H_5, R^1 = H), showed no antibacterial activity. Meanwhile, substitution with bulkier alkyl or aryl groups **3c, e-h** improved the anti-bacterial activity against most of the tested bacteria. Results of 1-benzyl derivatives, compounds **4a-h** Table 3, showed moderate to excellent antibacterial activity against all tested bacteria except **4h** was inactive against *P. aeroginosa*.

Also, it was observed that many of the tested compounds showed variable degrees of antibacterial activity against *S. marcescens*, while the reference drug was inactive. This can be explained on the basis that chloramphenicol resistance is most often manifested in *S. marcescens* due to its acetylation by chlora-mphenicol acetyl transferase enzyme.[29]

Antifungal activity[20]

Results of the antifungal activity indicated that most of the tested compounds did not show antifungal

activity, while compounds (**2a**) and (**4a**) showed 23% against *Candida albicans* and 53% against *Asperigillus flavus* of that of fluconazole, respectively.

B- Pharmacological screening

Anti-inflammatory activity

Compounds **2-4** were evaluated for their *in vivo* anti-inflammatory effects by the carrageenan induced paw edema bioassay in rats using indomethacin as a reference drug.[21] The results of the percentage of edema inhibition at a dose of 0.02 mmol/Kg and time intervals 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 h are cited in Table 4.

The results for compounds **2a-h** revealed that at **3** h interval most of the tested compounds exhibited 33-72% of the anti-inflammatory activity of indomethacin, while at 5 h interval compounds **2a-h** showed 20-33% the anti-inflammatory activity of indomethacin.

At 3 h time interval compounds **3a, c, e-h** showed 24-103% of the anti-inflammatory activity of indomethacin. In addition, compound **3g** was found to be the most potent member of this series and compound **3f** being the least active derivative. At 5 h interval only compound **3g** showed moderate anti-inflammatory activity (76% that of indomethacin).

The results for compounds **4a-h** revealed that at 3 h interval the tested compounds exhibited 40-112% of that of indomethacin, where compounds **4b**, **4c**, and **4g** showed comparable results to that of indomethacin (96, 95, and 95% of indomethacin), respectively, while compounds **4a** and **4h** supersedes that of indomethacin (103 and 112%), respectively. At 5 h interval only compounds **4a**, **4b**, **4e**, and **4h** having H, CH_3, *c*-C_6H_{11}, and *p*-Cl-C_6H_4 as substituents showed interesting anti-inflammatory activity (89, 88.5, 75.5, and 76% of that of indomethacin). Also, it was observed that replacement of the terminal phenyl group (compound **4f**), (33% of indomethacin) by *p*-tolyl or *p*-chlorophenyl (compounds **4g** and **4h**) markedly increases the activity (69 and 76%), respectively.

Analgesic activity

The most active anti-inflammatory compounds (**3g**, **4a**, **4b**, **4e**, and **4h**) and the reference drug were tested *in vivo* for their analgesic activity using mean licking time in the hot plate test[21] at 0.02 mmol/Kg dose level. Results in Table 5 revealed that all the tested compounds exhibited analgesic activity comparable to that of indomethacin and they have rapid onset of action at 0.5 h time interval, with compound **3g** being the most active one showing analgesic activity twice that of indomethacin. At 1 and 2 h time intervals the most active ones were compounds (**3g** and **4e**). Compounds carrying the *N*-benzyl group at indole nitrogen (**4a**, **4b**, **4e**, and **4h)** showed that at 5 h interval compound **4h** was the most active one giving comparable results to that of indomethacin that might be attributed

to a better fitting of the *p*-chlorophenyl group at the receptor.

Ulcerogenic effect[22]

Observation of the gastrointestinal mucosa for the presence of lesions following oral administration of graded doses (10, 30, and 50 mg/Kg) of the tested compound as well as the reference drug has been taken as an indication for the ulcerogenic effects.[22] Both the frequency of ulceration (expressed as ratio of ulcerated animals) and the severity of ulceration (expressed as ulcer index) were used for comparison of the tested compounds and indomethacin.

Compounds **4a**, **4b**, and **4h** that exhibited potent analgesic and anti-inflammatory profile in the pre-mentioned animal models were evaluated for their ulcerogenic effect in rats.[22] Results are recorded in Table 6.

The tested compounds **4b** and **4h** showed superior GI safety profile, since they gave 100% protection in the population of the tested animals at oral dose 10 mg/Kg, while at 30 and 50 mg/Kg they elicited 17% and 50% protection, respectively.

In addition, compound **4a** was found to cause 33%, 67%, and 100% ulceration, respectively when compared to indomethacin which showed 67% and 100% ulceration at 10 and 30 mg/Kg, respectively.

It should be noted that, compound **4h** was the most active derivative with regard to the anti-inflammatory and analgesic activities containing in its structure a benzyl group on indole nitrogen resembling the structure of indomethacin, in addition, to the bulky *p*-chlorophenyl group that might be responsible for the better fitting to the cyclooxygenase enzyme.[30] Therefore, the orientation of the compound **4h** in the cyclooxygenase active site was examined by a docking experiment.[31] This study showed that compound **4h** binds in the center of the primary binding sites of COX-2 enzyme with its *p*-chlorobenzyl group occupies the channel area and is stabilized by hydrophobic interaction with the amino acid residues Ile341, Phe361, Leu534, and Met535. In addition, the benzyl group interacts with the secondary pocket amino acid residues Leu352, Val523, and Trp387, Fig. 1.

Acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of compounds **4b** and **4h** was also determined in mice according to a reported method[23] and was found to be 250 and 300 mg/Kg (*i.p.*), whereas that of indomethacin equal to 13 mg/Kg (*i.p.*).[32]

CNS depressant activity

(i) Anticonvulsant activity

Blockade of pentylenetetrazole-induced convulsions in mice is a characteristic effect of some CNS depressant drugs. Compounds **2a-h** were investigated for their anticonvulsant activity by following the anticonvulsant drug development (ADD) program.[24] At a dose level of 2.8 mmol/Kg, (the effective dose determined from a dose response

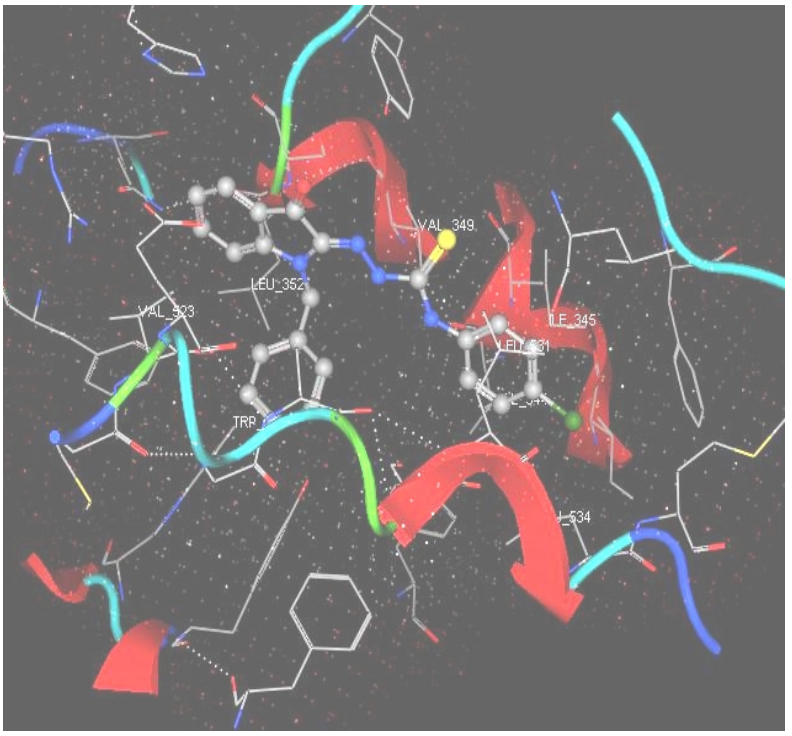


Fig. 1: The docking of compound **4h** in the active site of COX-2 enzyme.

curve) neither clonic, nor tonic convulsions have been observed for animals treated with either the test compounds (**2a-h**) or diazepam, Table 7. Reduction of the administered dose of the tested compounds (**2a-h**) to 2.0 mmol/Kg showed that 4 compounds; **2a** and **2c** elicited 75% protection against PTZ induced convulsions and compounds **2g** and **2h** 50% protection against convulsions, while compounds **2b**, **2d-f** and diazepam induced 100% protection. Further decrease of the administered dose to 1.4 mmol/Kg lead to 25% protection in case of compounds **2b** and **2d-f** while compounds **2a**, **2c**, **2g** and **2h** were inactive at this dose level and diazepam showed 100% protection. These results correlate well with the Clog P of these compounds, which ranged from 1.35 to 4.09 and with the fact that the crossing of the blood-brain barrier by CNS agents appears to be optimal at Clog P value of 2.0,[26] Table 8.

The dose level (1.4 mmol/Kg) was further used for testing anticonvulsant activity of compounds **3** and **4** (the Clog P values for these derivatives ranged from 1.68-4.43 and 2.83-5.57, respectively, Table 8). These compounds embody ethyl and benzyl

moieties at indole nitrogen atom and different alkyl or aryl moieties on terminal nitrogen of thiosemi-carbazide group. Selection of the ethyl or benzyl moieties based on the potent results shown above (compounds **2a-h**) and on the enhancement of lipophilic character of these compounds.

Comparison of the anticonvulsant activity of compounds (**3a, c, e-h**) with the corresponding lower homologues (**2a-h**) results in 20% increase of activity which is in a good agreement with Clog P of these compounds.[26] Also, study of results of compounds (**3f-h**) shows that substitution of Cl for the *p*-H of the phenyl group didn't alter the activity, while the *p*-CH_3 substituent increases the activity from 75% to 100% protection. This may be explained on the grounds of the better fitting of the *p*-tolyl containing compound than the other halogenated ones.

Also, replacement of methyl group of indole nitrogen of compound (**2a)** by a benzyl one, compound (**4a**) showed a dramatic increase in the anticonvulsant activity from 0.0 to 100% protection. This can be attributed to the higher lipid solubility of the benzyl moiety-containing compound (**4a**) in comparison to the methyl-containing one (**2a**). Data of anticonvulsant activity of compounds (**4a-h**) revealed that compounds **4a-d** and diazepam showed 100% protection while, compounds **4e** and **4f** showed 50% protection and compounds **4g** and **4h** showed 25% protection. The last four compounds were found to initiate epileptic fits in mice.

Therefore, these last four compounds (**4e-h**) were further tested for their anticonvulsant activity at a dose level of 1.0 mmol/Kg and they showed 100% protection. This can be explained according to the following facts that chlorpromazine in small dose is used as antiemetic and used clinically in small doses even in epileptic patients, but in higher doses (antipsychotic action) it is contraindicated in patients with epileptic psychosis because it potentiate epileptic fits.[32]

Hence, we can conclude that the target compounds with low lipophilicity have protective effect due to the inhibitory action on the sub cortical region (brain) so; the synthesized compounds have antiepileptic activity. By increasing the lipophilicity of the compounds which means rapid crossing to the BBB and presence in high concentration will induce epileptic activity by affecting the motor area in the cerebral cortex.[33]

ii) The rota-rod motor coordination test

Rolling roller performance (RRP) test was used for the evaluation of any neurological deficit (e.g. ataxia, sedation or hyperexcitability).[25] The ability of mice to remain on a rotating rod for 20 seconds without falling was determined at 10 min intervals over a period of 2 h. Results revealed that the tested compounds **2- 4** do not have any depressant effect on motor

system as they gave no ataxia (loss of coordination) and the mice can preserve their position on rota-rod test as control while, diazepam induce positive rota-rod test due to its central inhibitory action on motor system.

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