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Eco-friendly synthesis, characterization, and drug-likeness properties of new uracils and their biological evaluation

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Abstract: Several pyridopyrimidine derivatives have been synthesized through different synthetic processes. The different physicochemical factors for their synthesis were also discussed. A comparison between the conventional and microwave-assisted synthesis was conducted by comparing total time of reaction and its yield percentage. Most of the produced uracils were established for their anti-inflammatory, analgesic, and antioxidant activities. Compounds **1** and **3** were demonstrated as the best results against DNA damage, whereas compounds **2a** and **2b** exhibited an effective anti-inflammatory activity. Moreover, the Lipinski rule parameters were calculated for the synthesized compounds. The results indicated that compounds **1**, **2a** and **5** have good potential for subsequent development bioactivity.

Keywords: biological activity; conventional; drug-likeness; microwave; pyridopyrimidine.

Abbreviations

ABS %	Absorption percentage
ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid
AcOH	Glacial acetic acid
Da	Dalton
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
EM	Electron multiplier
EtOH	Ethanol
H-NMR	Hydrogen nuclear magnetic resonance
HBA	Hydrogen bond acceptor
HBD	hydrogen bond donor
IR	Infrared
Log P	Lipophilicity
MP	Melting point
m/z	Mass to charge ratio
M^+	Molecular ion
milog	Molinspiration predicted log P
MORE	Microwave-induced organic reaction enhancement
MS-IE	Mass spectroscopy – electronic ionization
MW	Microwave
MW	Molecular weight
noHNH	Number of hydrogen bond donor
noN	Number of hydrogen bond acceptor
nrotb	Number of rotatable bond
nviol	Number of violations
$POCl_3$	Phosphorous oxychloride
PSA	Polar surface area
TMS	Tetramethylsilane

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1 Introduction

Compounds with fused heterocyclic system are of great importance because these structures occur in many products, such as antibiotics, hormones, pharmaceuticals, alkaloids, vitamins, dyes, and herbicides [1, 2]. The pyridopyrimidine derivatives can be prepared by many routes to create derivatives with diverse pharmacological activities, including anti-inflammation [3], anti-bacterial [4], analgesic [5], and antimalarial activities [6]. Meanwhile, microwave-induced organic reaction enhancement ("MORE") is a branch of green chemistry that has focused on rapid organic synthesis [7–9]. The main target of MORE is to offer simple, economical, ecologically friendly, and improved reaction rate. Some computational approaches have been proposed to rationalize experiments by focusing on compounds that are more likely to have the desired activity and bioavailability. Due to the high reactivity of the pyridopyrimidine derivatives and as an extension of our previous works [10–14], herein, we reported the synthesis of new pyridopyrimidines using microwave irradiation, with the aim of improving the reaction rate, yield, and time related compared with the conventional method. In addition, their biological evaluations as antioxidant, anti-tumor, and anti-inflammatory agents were discussed.

2 Materials and methods

2.1 Instruments

The Gallenkamp apparatus was used to record all melting points, and the IR-spectra were obtained using Perkin Elmer Infrared Spectrophotometer Model 157, Grating (KBr; ν/cm^{-1}). The 1H-NMR result were obtained using the Varian (300; 75 MHz) spectrophotometer (TMS; δ/ppm) as an internal reference and the DMSO-d_6 as a solvent. The MS-EI were recorded on 70 eV with the Varian MAT 311Kratos. The elemental analyses were carried out at the micro-analytical center of Cairo University, Giza, Egypt. Results were in good agreement (±0.3%) with the calculated values. Irradiation was performed in a domestic MW oven (E. M-230 M; 800 W). All spectral data were illustrated in Table 1. All chemicals are from Sigma-Aldrich; Molinspiration.com (http://molinspiration.com) is a chemical informatics software vendor based in Slovakia.

2.2 Chemistry

General method for the microwave-assisted synthesis (Method B): The reactants used in method A (conventional method) were placed in a closed Teflon vessel, and were irradiated in microwave at 800 W (see reaction times, Table 2). The reaction mixture was treated in the same way as the conventional method (Method A), thereby obtaining the desired compound.

2.2.1 Synthesis of 8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-2-thioxo-1,2,3,6,7,8-hexahydro-4H-cyclopenta[5,6] pyrido[2,3-d] pyrimidin-4-one (1): Method (A): A multicomponent reaction by the reaction of *p*-anisaldehyde (0.04 mol), 6-aminothiouracil (0.02 mol), and cyclopentanone (0.02 mol) in (10 ml) DMF was refluxed for 8 h. The precipitate obtained was crystallized from DMF to produce compound **1** as pale yellow crystals.

2.2.2 Synthesis of 2-alkyl thio derivatives 2a–e: Method A: Compound **1** (0.02 mol) was dissolved in a warmed solution of alcoholic KOH (15 ml), which was warmed for 30 min and then cooled. To this, the halogenated-compound (0.02 mol) was added with stirring under reflux for 5 h. The solid produced crystals from ethanol to afford compounds **2a–e**.

2.2.2.1 Synthesis of 8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-2-(methylthio)-3,6,7,8-tetrahydro-4H-cyclopenta[5,6]pyrido [2,3-d]pyrimidin-4-one (2a)

This was produced from 0.02 mol of compound **1** and 0.02 mol methyl iodide as pale yellow crystals and recrystallized from dioxane.

2.2.2.2 Synthesis of 2-(ethylthio)-8-(4-methoxybenzyli dene)-5-(4-methoxyphenyl)-3,6,7,8-tetrahydro-4H-cyclopenta[5,6] pyrido[2,3-d]pyrimidin-4-one (2b)

This was produced from 0.02 mol of compound **1** and 0.02 mol of ethyliodide as deep yellow crystals and recrystallized from DMF.

2.2.2.3 Synthesis of 2-(hexadecylthio)-8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-3,6,7,8-tetrahydro-4H-cyclopenta[5,6] pyrido[2,3-d]pyrimidin-4-one (2c)

This was produced from 0.02 mol of compound **1** and 0.02 mol of 1-chloropentadecane as orange crystals and recrystallized from DMF.

2.2.2.4 Synthesis of 8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-2-((2-oxopropyl)thio)-3,6,7,8-tetra hydro-4H-cyclopenta[5,6] pyrido[2,3-d]pyrimidin-4-one (2d)

This was produced from 0.02 mol of compound **1** and 0.02 mol chloroacetone as yellow crystals, which was crystallized from ethyl alcohol.

2.2.2.5 Synthesis of 8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-2-((2-oxo-2-phenylethyl)thio)-3,6,7,8-tetrahydro-4H-cyclopenta[5,6]pyrido[2,3-d] pyrimidin-4-one (2e)

It was produced from 0.02 mol of compound **1** and 0.02 mol phenacylbromide as yellow crystals and recrystallized from ethyl alcohol.

2.2.3 Synthesis of 8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-3-methyl-2-(methylthio)-3,6,7,8-tetrahydro-4H-cyclopenta[5,6] pyrido[2,3-d]pyrimidin-4-one (3): Method A: Compound **2a** (0.02 mol) was dissolved in sodium ethoxide (20 ml), after which the mixture was heated for 30 min and then cooled. The alkyl-iodide (0.02 mol) was added and then refluxed with stirring for 3 h. The solid obtained was crystallized from ethyl alcohol to afford compound **3** as brown crystals.

2.2.4 Synthesis of 5-aryl-2-hydrazino-9-arylmethyl ene-6,7,8,9,-tetrahydropyrimido[4,5-b]quinolin-4-one (4): Method A: Compound **4** was obtained from a refluxing a solution of compound **2a** or **2b** (0.02 mol) in hydrazine-hydrate (20 ml) for 8 h. The solid obtained after cooling was separated and crystallized using DMF as yellow crystals.

2.2.5 Synthesis of 8-(4-methylbenzylidene)-2-(methylsulfonyl)-5-(p-tolyl)-3,6,7,8-tetrahydro-4H-cyclopenta[5,6]pyrido[2,3-d] pyrimidin-4-one (5): Method A: Compound **2a** (0.02 mol) was heated with stirring for 10 h with excess hydrogen-peroxide (6 ml) in glacial acetic acid (15 ml). After cooling, the obtained solid was filtered and crystallized from DMF to give compound **5** as white powder.

2.2.6 Synthesis of 4-chloro-8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-2-(methyl thio)-4,6,7,8-tetrahydro-3H-cyclopenta[5,6]pyrido [2,3-d]pyrimidine (6): Method A: Compound **2a** (0.02 mol) and 7 ml of phosphorus oxychloride were refluxed with stirring for 6 h in 30 ml dry dioxane. The mixture was cooled and poured onto crushed ice, after which the produced solid was separated and crystallized from ethyl alcohol to produce compound **6** as yellow powder.

2.2.7 Synthesis of 8-(4-methoxybenzylidene)-N,5-bis(4-methoxyphenyl)-2-(methylthio)-7,8-dihydro-6H-cyclopenta[5,6]pyrido [2,3-d]pyrimidin-4-amine (7): Method A: *p*-anisidine (0.02 mol) was added to compound **6** (0.02 mol) in 15 ml acetic acid, after which it was refluxed with stirring for 4 h. The produced solid was collected and recrystallized using DMF to afford product **7** as yellow crystals.

2.2.8 Synthesis of compounds 8a,b: Method A: Compound **6** (0.02 mol) was refluxed with stirring for 4 h with piperazine or morpholine (0.02 mol) in glacial acetic acid (15 ml). The obtained

Table 1: Spectral data for compounds **1–11.**

Compound no.	IR-spectroscopy (KBr, cm^{-1})	^{1}H-NMR spectra (DMSO-d_6, δ, ppm)	Mass spectra m/z (%)
1	3321, 3096 (2 NH), 3025 (CH-aryl), 2921 (CH-alkyl), 1682 (C=O), 1631 (C=N), 1370 (C=S)	2.29 (2H, t, CH_2), 2.99 (2H, t, CH_2), 3.83 (6H, s, 2OCH_3), 4.0 (1H, s, NH), 6.82–7.81 (8H, m, Ar-H), 8.13 (1H, s, NH), 12.33 (1H, s, NH)	443 M^+, 421 (54), 305 (63), 236 (22), 204 (27)
2a	3403 (NH), 3036 (CH-aryl), 2930 (CH-alkyl), 1680 (C=O), 1647 (C=N)	2.29 (2H, t, CH_2), 2.49 (3H, s, SCH_3), 2.99 (2H, t, CH_2), 3.81 (6H, s, 2OCH_3), 6.94–7.60 (8H, m, Ar-H), 8.13 (1H, s, CH), 11.50 (1H, s, NH)	455 $(M-2)^+$ (48), 356 (38), 236 (42), 219 (28), 144 (31), 99 (100)
2b	3385 (NH), 3059 (CH-aryl), 2912 (CH -alkyl), 1696 (C=O), 1620 (C=N)	1.08 (3H, t, CH_3), 2.29 (2H, t, CH_2), 2.99 (2H, t, CH_2),3.41 (2H, q, SCH_2), 3.78 (6H, s, 2OCH_3), 6.83–7.18 (8H, m, Ar-H), 8.77 (1H, s, CH), 12.22 (1H, s, NH)	471 M^+ (82), 425 (78), 316 (80), 177 (68), 108 (100)
2c	3398 (NH), 3086 (CH-aryl), 2899 (CH-alkyl), 1698, 1680, 1673, (3C=O), 1629 (C=N)	0.88 (3H, t, CH_3), 1.26–1.90 (28H, m, $(CH_2)_{14}$), 3.24 (2H, t, SCH_2),3.81 (6H, s, 2OCH_3), 6.83–7.82 (8H, m, Ar-H), 8.15 (1H, s, CH), 11.52 (1H, s, NH); (br, NH, D_2O exchangeable)	667 M^+ (19), 235 (82), 192 (12), 135 (21), 77 (74)
2d	396 (NH), 3081 (CH aryl), 2910 (CH-alky), 1696, 1677 (2C=O), 16 (C=N)	2.28 (3H, s, $COCH_3$), 2.41 (2H, t, CH_2), 2.97 (2H, t, CH_2), 4.03 (2H, s, SCH_2), 6.83 (1H, s, NH), 6.72–7.15 (8H, m, Ar-H)	499 $(M-2)^+$ (78), 437 (68), 317 (62), 262 (58), 198 (52)
2e	3398 (NH), 3063 (CH-aryl), 2935 (CH-alkyl), 1705, 1689 (2C=O), 1639 (C=N), 1376 (C=S)	2.43 (2H, t, CH_2), 2.99 (2H, t, CH_2), 3.81 (6H, s, 2OCH_3), 4.84 (2H, s, SCH_2), 7.13–7.87 (13H, m, Ar-H), 8.12 (1H, s, CH), 11.50 (1H,s, NH)	559 $(M-2)^+$ (28), 454 (78), 382 (24), 315 (75), 197 (75), 77 (100)
3	3067 (CH-aryl), 2926 (CH-alkyl), 1685 (C=O), 1632 (C=N)	2.28 (2H, t, CH_2), 2.59 (3H, s, NCH_3), 2.86 (2H, t, CH_2), 3.45 (3H, s, SCH_3) 3.83 (6H, s, 2OCH3), 6.89–7.60 (8H, m, Ar-H)	472 $(M+1)^+$ (48), 452 (18), 327 (24), 196 (58), 99 (100)
4	3186 (NH), 3024 (CH-aryl), 2921 (CH-alkyl), 1682 (C=O), 1669 (C=N)	2.41 (2H, t, CH_2), 2.89 (2H, t, CH_2), 5.18 (2H, s, NH_2), 6.83 (1H, s, NH), 6.82–7.85 (8H, m, Ar-H), 8.12 (1H, s, NH)	443 $(M+2)^+$ (78), 387 (68), 263 (58), 149 (72), 90 (92)
5	3402 (NH), 3028 (CH-aryl), 2924 (CH-alkyl), 1627 (C=N), 1159, 13,556 (SO_2)	2.28 (2H, t, CH_2), 2.99 (2H, t, CH_2), 3.30 (3H, s, SO_2-CH_3), 3.81 (6H, s, 2OCH_3), 6.94–7.60 (8H, m, Ar-H), 8.15 (1H, s, =CH), 11.47 (1H, s, NH)	489 M^+ (20), 425 (26), 397 (27), 304 (100), 271 (32), 155 (27)
6	3049 (CH-aryl), 2899 (CH-alkyl), 1621 (C=N), 1159 (C-C1)	2.29 (2H, t, CH_2), 2.50 (3H, s, SCH_3), 2.99 (2H, t, CH_2), 3.80 (6H, s, 2OCH_3), 6.94–7.60 (8H, m, Ar-H), 8.13 (1H, s, CH)	475 M^+ (42), 391 (38), 301 (100), 231 (88), 143 (48)
7	3398 (NH), 3048 (CH-aryl), 2909 (CH-alkyl), 1627 (C=N)	2.29 (2H, t, CH_2), 2.44 (3H, s, SCH_3), 2.99 (2H, t, CH_2), 3.81 (9H, s, 3OCH_3), 6.94–7.60 (12H, m, Ar-H), 7.50 (1H, s, NH), 8.13 (1H, s, CH)	562 M^+ (58), 454 (56), 316 (60), 211 (67), 167 (78), 65 (100)
8a	3401 (NH), 3045 (CH-aryl), 2918 (CH-alkyl), 1621 (C=N)	2.30 (2H, t, CH_2), 2.44 (3H, s, SCH_3), 2.99 (2H, t, CH_2), 2.58–3.20 (8H, m, 4CH_2), 3.81 (6H, s, 2OCH_3), 6.94–7.60 (8H, m, Ar-H), 8.13 (1H, s, CH)	525 M^+ (58), 487 (56), 432 (87), 211 (67), 167 (78), 98 (100)
8b	3293 (NH), 3054 (CH-aryl), 2936 (CH-alkyl), 1632 (C=N)	2.29 (2H, t, CH_2), 2.44 (3H, s, SCH_3), 2.82 (2H, t, CH_2), 2.89–3.20 (8H, m, 4CH_2), 3.81 (6H, s, 2OCH_3), 6.94–7.60 (8H, m, Ar-H), 8.13 (1H, s, CH)	525 M^+ (58), 487 (56), 432 (87), 211 (67), 167 (78), 98 (100)
9	3510 (OH), 3368 (NH), 3037 (CH-aryl), 2912 (CH-alkyl), 1716 (C=O), 1626 (C=N)	2.29 (2H, t, CH_2), 2.44 (3H, s, SCH_3), 2.99 (2H, t, CH_2), 3.81 (6H, s, 2OCH_3), 6.94–7.60 (12H, m, Ar-H), 7.71 (1H, s, NH), 8.13 (1H, s, CH), 13.11 (1H, s, OH)	575 $(M-1)^+$ (22), 512 (17), 478 (45), 355 (65), 264 (21), 98 (100)
10	3016 (CH-aryl), 2932 (CH-alkyl), 1708 (C=O), 1625 (C=N)	2.29 (2H, t, CH_2), 2.59 (3H, s, SCH_3), 2.99 (2H, t, CH_2), 3.81 (6H, s, 2OCH_3), 6.94–7.70 (12H, m, Ar-H), 8.13 (1H, s, CH), 13.11 (1H, s, OH)	559 $(M-1)^+$ (22), 512 (17), 478 (45), 355 (65), 264 (21), 98 (100)
11	3350 (NH), 3025 (C- aryl), 2936 (CH-alkyl), 1638 (C=N)	2.29 (2H, t, CH_2), 2.99 (2H, t, CH_2), 3.81 (6H, s, 2OCH_3), 4.50 (1H, s, CH), 4.77 (2H, s, NH_2), 6.94–7.60 (12H, m, Ar-H), 7.79 (2H, s, NH_2), 8.15 (1H, s, NH), 9.39 (1H, s, NH)	455 M^+ (22), 355 (65), 264 (21), 98 (100)

Table 2: The physical constants of the pyridopyrimidine derivatives **1–11.**

No.	Molecular formula	Molecular weight	m.p. (°C)	Conventional method			Microwave method		
				Yield (%)	Time (h)	Solvent	Yield (%)	Time (min)	Solvent
1	$C_{25}H_{21}N_3O_3S$	443	315–320	60	8	DMF	89	10	DMF
2a	$C_{26}H_{23}N_3O_3S$	457	>300	73	5	KOH + EtOH	89	6	KOH + EtOH
2b	$C_{27}H_{25}N_3O_3S$	471	>300	58	5	KOH + EtOH	83	6	KOH + EtOH
2c	$C_{41}H_{53}N_3O_3S$	667	290–295	68	5	KOH + EtOH	82	6	KOH + EtOH
2d	$C_{28}H_{25}N_3O_4S$	499	310–315	52	5	KOH + EtOH	79	6	KOH + EtOH
2e	$C_{33}H_{27}N_3O_4S$	561	270–275	63	5	KOH + EtOH	88	6	KOH + EtOH
3	$C_{27}H_{25}N_3O_3S$	471	300–305	64	3	NaOEt	79	10	NaOEt
4	$C_{25}H_{23}N_5O_3$	441	>300	50	8	HYDRAZINE	80	10	–
5	$C_{26}H_{23}N_3O_5S$	489	295–300	54	10	$AcOH + H_2O_2$	80	15	$AcOH + H_2O_2$
6	$C_{26}H_{22}ClN_3O_2S$	475	310–315	70	6	Dioxane + $POCl_3$	86	8	Dioxane + $POCl_3$
7	$C_{33}H_{30}N_4O_3S$	562	270–275	73	4	AcOH	86	10	AcOH
8a	$C_{30}H_{31}N_5O_2S$	525	292–296	71	5	AcOH	88	8	AcOH
8b	$C_{30}H_{30}N_4O_3S$	526	280–285	75	5	AcOH	86	8	AcOH
9	$C_{33}H_{28}N_4O_4S$	576	>300	60	4	AcOH	82	7	AcOH
10	$C_{33}H_{28}N_4O_3S$	560	295–300	62	8	$AcOH + H_2SO_4$	74	10	$AcOH + H_2SO_4$
11	$C_{25}H_{25}N_7O_2$	455	280–285	75	12	EtOH	85	12	EtOH

solid was crystallized from dioxane to produce compounds **8a** and **b**.

2.2.8.1 Synthesis of 8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-2-(methylthio)-4-(piperazin-1-yl)-7,8-dihydro-6H-cyclopenta[5,6]pyrido[2,3-d]pyrimi dine (8a)
These were produced as yellow crystals.

2.2.8.2 Synthesis of 4-(8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-2-(methylthio)-7,8-dihydro-6H-cyclo penta[5,6] pyrido[2,3-d]pyrimidin-4-yl)morpholine (8b)
These were produced as yellow crystals.

2.2.9 Synthesis of 2-((8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-2-(methylthio)-7,8-dihydro-6H-cyclopenta[5,6]pyrido [2,3-d]pyrimidin-4-yl)amino) benzoicacid (9): Method A: Compound **6** (0.02 mol) and anthranilic acid (0.02 mol) were refluxed with stirring for 4 h in glacial acetic acid (15 ml). The solid produced was separated and recrystallized using dioxane to produce compound **9** as yellow powder.

2.2.10 Synthesis of 3-(4-methoxybenzylidene)-14-(4-methoxyphenyl)-6-(methylthio)-2,3-dihydrocyclopen ta[5′,6′]pyrido[2′,3′:4,5]pyrimido [6,1-b]quinazolin-8(1H)-one (10): Method A: Compound **9** (0.02 mol) was refluxed with stirring for 8 h in glacial acetic acid (15 ml) in the presence of small amount of sulfuric acid (1 ml). The mixture was quenched by ice water and neutralized by ammonia solution, after which the solid produced was collected and crystallized from DMF to give a product **10** as yellow crystals.

2.2.11 Synthesis of 2,4-dihydrazinyl-8-(4-methoxybenzylidene)-5-(4-methoxyphenyl)-7,8-di hydro-6H-cyclopenta[5,6]pyrido[2,3-d] pyrimidine (11): Method A: Compound **6** (0.02 mol) was stirred under reflux in 5 ml ethanol and 20 ml dioxane with 10 ml hydrazine hydrate for 12 h. The obtained solid after cooling was collected and recrystallized using dioxane as yellow crystals.

2.3 Antioxidant activity

2.3.1 ABTS-assay: The test for the anti-oxidant activity was performed by following the Torres [15] and Pellegrini [16] techniques.

2.3.2 Erythrocyte hemolysis: The test for erythrocyte hemolysis was performed by using the Morimoto technique [17].

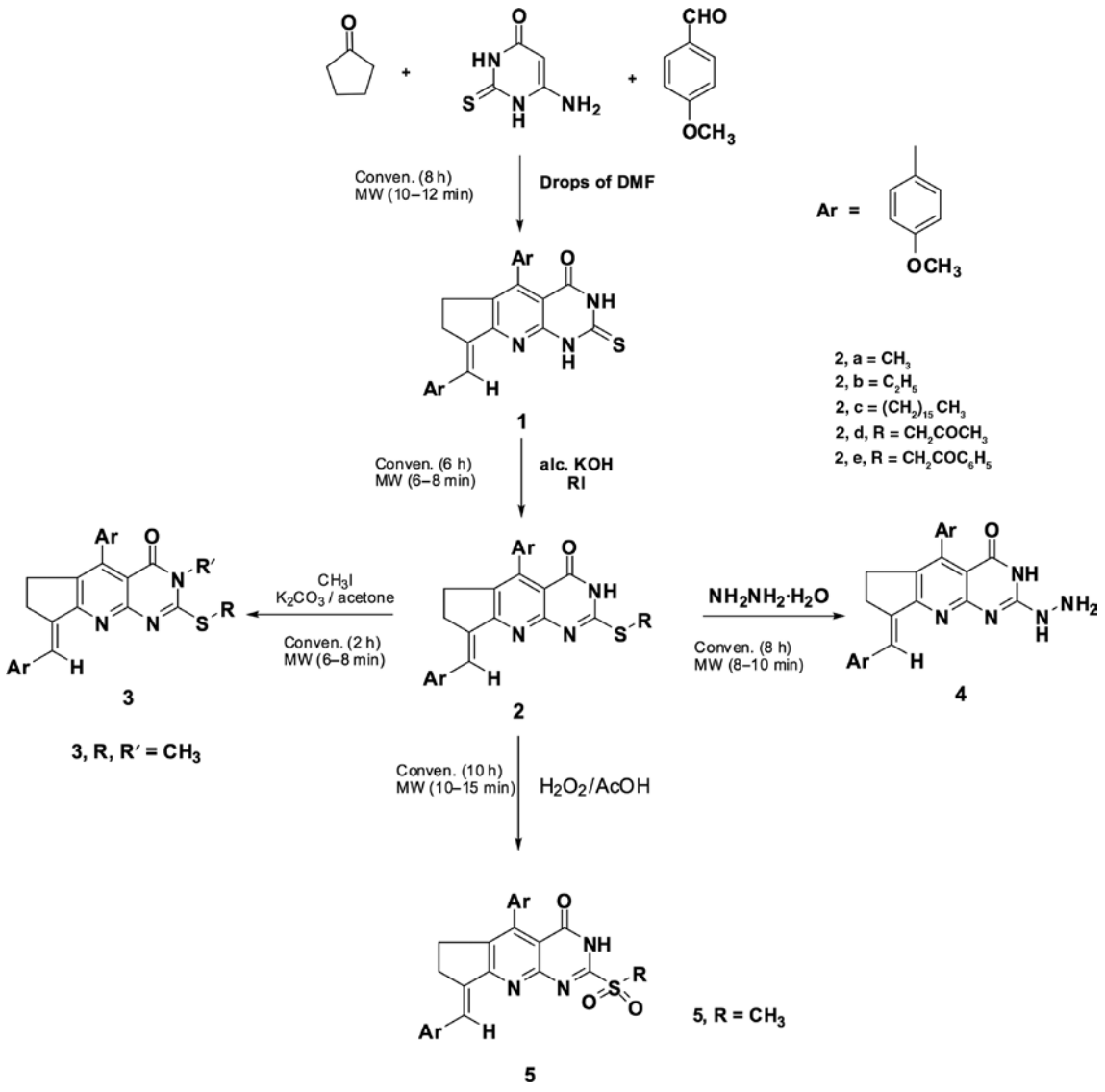
2.3.3 Bleomycin DNA damage dependent assay: This test was performed by following the methods proposed by Gutteridge [18] and Gimenez [19].

2.3.4 Anti-inflammatory carrageenan-induced rat hind paw edema model: This test was performed by following the method proposed by Winter [20].

3 Results and discussion

3.1 Chemistry

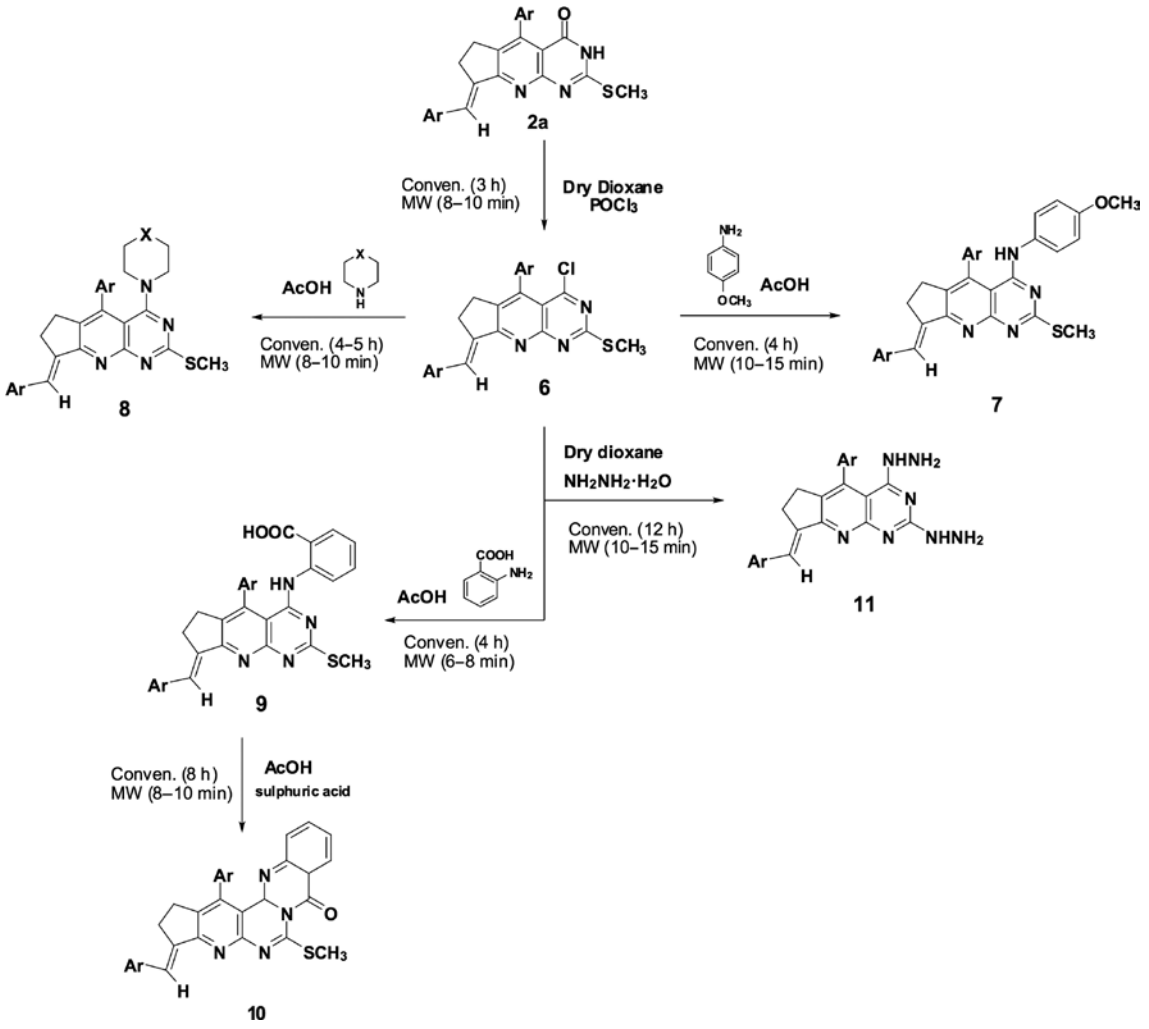
The 11 pyrido[2,3-d]pyrimidine derivatives existing in this work were synthesized through two synthetic routes (conventional and microwave-assisted methods), as illustrated in Schemes 1 and 2, respectively. The starting compound **1** was synthesized by the multicomponent reaction between 6-aminothiouracil, cyclopentanone, and *p*-anisaldehyde. The IR spectrum of **1** exhibited good absorbance rates ($\lambda = 3321, 3096\ cm^{-1}$) due to both NH groups being in compound **1**. On the one hand, the ^{1}H-NMR spectrum of compound **1** displayed four singlet signals ($\delta = 3.83, 4.0, 8.13, 12.33$ ppm), which can be attributed to the OCH_3,



Scheme 1: Reaction of pyridopyrimidine derivative **1** with different reagents under microwave irradiation.

N_3-H, CH olefinic, and N_1-H protons, respectively. Furthermore, it showed multiplet signals (δ = 6.82–7.81 ppm) due to the aromatic protons. The 2-alkyl thio derivatives **2a–c** were prepared through the reactions of compound **1** with different alkyl halides (Scheme 1). All structures **2a** – were proven by both elemental and spectral data. The ^{1}H-NMR spectrum exposed a singlet signal (δ = 2.50 ppm) equivalent to the SCH_3 protons.

On the other hand, the ^{1}H-NMR spectrum of compound **2b** indicated triplet and quartet signals (δ = 1.08, 3.41 ppm) owing to the CH_3 and SCH_2 protons of SC_2H_5 protons, respectively. Additionaly, compound **2c** showed triplet signals (δ = 0.88 ppm) in its ^{1}H-NMR spectrum corresponding to the terminal CH_3 of S-C_2H_5 and another one (δ = 3.24 ppm) due to SCH_2. Furthermore, the multiple signals (δ = 1.25–1.90 ppm) observed were attributable to the alkyl chain (-$C_{14}H_{28}$) protons. The reaction of **1** with α-haloketone, namely, chloro-acetone or phenacyl-bromide, gave compounds **2d** and **e**. The ^{1}H-NMR spectrum of **2d** displayed two singlet signals (δ = 2.28, 4.03 ppm) equivalent to CH_3 and CH_2 of chloroacetonyl moiety, respectively. Compound **2e** displayed a singlet signal (δ = 4.84 ppm) due to the SCH_2 protons. Compound **4** was obtained through the reaction of compounds **2a** and **b** with hydrazine-hydrate. Compound **4** revealed singlet signals (δ = 5.18 ppm) in its ^{1}H-NMR spectrum owing to the NH_2 protons and δ = 6.83 ppm corresponding to the NH proton. Meanwhile, further alkylation at the N-3 of compound **2a** when it was treated with methyl-iodide afforded compound **3**. Compound **3** exhibited two singlet signals



Scheme 2: The reaction of thiomethyl derivative **2a** with different amines.

(δ = 2.59, 3.45 ppm) attributable to the N-CH_3 and S-CH_3 protons in its ^{1}H-NMR spectrum. The oxidation of compound **2a** with hydrogen peroxide in glacial acetic acid afforded 2-methylsulfone derivative **5**. The ^{1}H-NMR spectrum exposed a singlet signal (δ = 3.3 ppm) owing to the 2-methyl sulphone protons.

Additionally, compound **6** was synthesized from the reaction of **2a** in dry dioxane with phosphorus oxychloride. The ^{1}H-NMR spectrum of compound **6** displayed a singlet signal (δ = 2.50 ppm) due to the S-CH_3 protons. Furthermore, the nucleophilic reactions of compound **6** towards different amines, namely, *p*-anisidine, piperazine, morphiline, and hydrazine-hydrate were investigated to produce compounds **7**, **8a**, **8b**, **9** and **11**, respectively. Two singlet signals (δ = 2.44, 8.13 ppm) appeared in the ^{1}H-NMR spectrum of compound **7** owing to the SCH_3 and NH protons, respectively. The mass spectra of compounds **8a** and **8b** exhibited the ion peaks (m/z = 525 M^+, 526 M^+) conforming to the molecular formula of both. Meanwhile, compound **6** reacted with anthranilic acid producing compound **9**. The IR spectrum of compound **9** revealed absorption bands at (λ = 3510, 3368, 1716 cm^{-1}) owing to NH, OH, and CO, respectively. The ^{1}H-NMR spectrum exhibited five singlet signals (δ = 2.44, 3.81, 7.71, 8.13, and 13.11 ppm) attributable to the SCH_3, two OCH_3, NH, CH= olefinic and OH protons, respectively. Additionaly, compound **9** underwent cyclization in the presence of AcOH/H_2SO_4 to give the quinazolinone derivative **10**. The IR spectrum of compound **10** revealed the absorption band (λ = 1708 cm^{-1}) due to the CO function. The ^{1}H-NMR spectrum also displayed a singlet signal (δ = 2.59 ppm) attributable to the SCH_3 protons. The absence of the carboxylic proton signal (δ = 13.11 ppm) in compound **9** confirmed the cyclization reaction. The treatment of compound **6** with hydrazine hydrate in dry dioxane gave the dihydazinyl derivative **11.** The four singlet signals that appeared

in the ^{1}H-NMR spectrum of compound **11** (δ = 4.50, 4.77, 8.15, 10.12 ppm) were due to two NH_2 and two NH protons, respectively. Furthermore, the mass spectrum revealed the molecular ion peak (m/z = 455 M^+) attributable to the formula $C_{25}H_{25}N_7O_2$ (Scheme 2).

3.2 Biological properties

The products were also tested for compliance to the Lipinski rule of five, and were summarized in Table 3. A molecule is likely to be established as an orally active drug candidate when it obeys the following rules: (a) the hydrogen bond donor's number "HBD" should be ≤5, (b) the hydrogen bond acceptor's number "HBA" should be ≤10, (c) its molecular weight should be ≤500 Da, and (d) it should not have an octanol-water partition coefficient (≤5) [21]. The absorption degree is stated by the (%) of absorption, which is computed from the following equation: ABS % = 109 – (0.345 TPSA) [22, 23]. The scores of all compounds were calculated using the online software [24, 25]. The hydrogen bond donor and acceptor in the synthesized uracils obey the Lipinski rule of five, and thus some of the synthesized compounds have good permeability and absorption properties through the biological membrane. Dissolution is dependent on the influences of aqueous solubility, lipo-philicity (log P), and ignitability (p Ka). The log p values of compounds **1** and **2a**–**e** (Scheme 1) were between 4.53 and 9.99.

Additionally, the molecular weight played an important role in the drug action: if it is higher than the accepted limit, the bulkiness also increases, subsequently affecting the drug action (the interaction between the drug receptor and DNA). The molecular weights of compounds **1** and **2a**–**e** between 443.50 and 667.96 Da showed that compounds **1** and **2a** follow Lipinski's rule of five. Consequently, further reactions have been processed for compound **2a** to produce compounds **3**, **4** and **5** (Scheme 1). Therefore, the results revealed that compounds **4** and **5** follow Lipinski's rule of five.

Similarly, compound **1** reacted with $POCl_3$ to give compound **6** with one violation. Consequently, compound **6** was conducted for further substitution reactions to produce compounds **7–11** (Scheme 2). The log

Table 3: The calculated absorption rates (ABS %), polar surface area (PSA), Lipinski parameters[a], and drug-likeness model scores of the title compounds **1–11**.

Entry	Acceptable range	ABS % –	Nviol –	milog P ≤5	MW ≤500	noN ≤10	noHNH ≤5	TPSA –	nrotb –	Drug-likeness model score –
1		81.40	0	4.67	443.53	6	2	80.0	4	+0.70
2a		83.90	0	4.99	459.50	6	2	72.48	5	+0.75
2b		82.39	1	6.53	471.58	6	1	77.11	6	+0.84
2c		82.39	2	9.99	667.96	6	1	77.11	20	+0.78
2d		76.50	1	5.70	499.59	7	1	94.19	7	+1.04
2e		76.50	2	7.30	561.66	7	1	94.19	8	+1.16
3		86.14	1	5.67	471.58	6	0	66.26	5	+0.59
4		69.26	0	4.24	441.49	8	4	115.16	5	+1.16
5		70.61	0	4.47	489.55	8	1	111.26	5	+0.77
6		89.28	1	7.51	476.00	5	0	57.14	5	+0.30
7		81.94	2	8.63	562.70	7	1	78.41	8	+0.64
8a		84.01	2	6.23	525.68	7	1	72.41	6	+0.58
8b		84.98	2	6.78	526.66	7	0	69.62	6	+0.46
9		72.21	2	8.62	576.68	8	2	106.47	8	+1.44
10		82.64	2	5.83	560.68	7	0	76.40	5	+0.46
11		63.03	1	4.16	455.52	9	6	133.24	6	+0.71

[a]nviol, no. of violations; milog, molinspiration predicted log P; MW, molecular weight; noN, no. of hydrogen bond acceptor, noHNH, no. of hydrogen bond donor; and nrotb, no. of rotatable bond.

These properties are calculated and discussed on the basis of Lipinski's rule and its components. The compounds **2a**, **4** and **5** fulfill Lipinski's rule and show good drug likeness scores (Table 3). Milog P of these compounds was found below **5** meaning that these show good permeability across cell membrane. TPSA below 160 Å2, n violation = 0, No. hydrogen bond donors ≤5 (The sum of OHs and NHs), No. hydrogen bond acceptor ≤10 (The sum of Os and Ns). For organic molecules the probability is if the bioactivity score is (>0), then it is active, if (–5.0–0.0) then moderately active, if (<–5.0) then inactive. Compounds **1–11** were taken for further calculation of bioactivity score. From Table 3 Compounds **2a**, **2c**, **2e**, **4**, **5** and **7** showed good bioactivity score. Compound **4** showed good drug likeness score and bioactivity score, in comparison with other compounds.

p values for these compounds were between 5.83 and 8.63. Their molecular weights were between 525.68 and 576.68 with more than one violation. PSA is the sum of all surfaces of polar atoms presented in a molecule. The prediction of drug transport property depends mainly on the PSA, which is inversely proportional to the absorption %. Compounds like **3** and **6** have the least polar surface areas among the series so they have the maximum absorption. Meanwhile, compounds **1**, **2a**, **4** and **5** exhibited violations of the discussed criteria (Table 3). Hence, these compounds have a good potential for eventual development as oral agents and can be potentially active drug candidates. Although the other compounds showed a narrow therapeutic margin, there is chemical space to design and develop more selective and potent compounds.

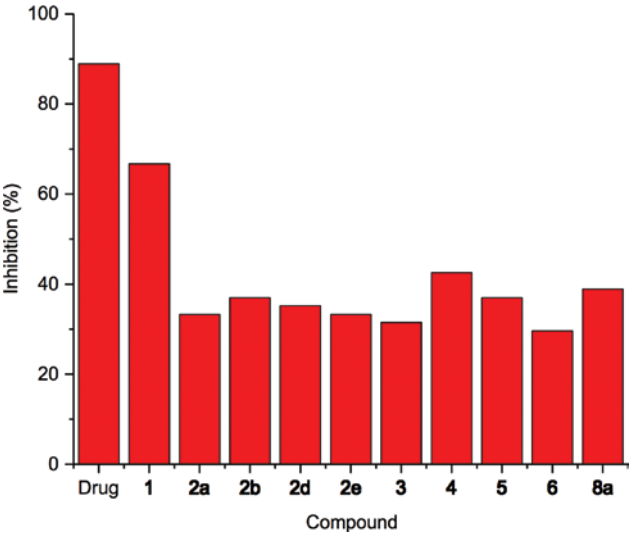


Figure 2: ABTS (inhibition %).

3.3 Biological activity

3.3.1 Antioxidant activity for erythrocytes hemolysis and ABTS

Most of the synthesized pyridopyrimidine derivatives were examined for their antioxidant activity using ABTS assay. The results showed their ability to inhibit lipid peroxidation in rat kidney and brain homogenates along with the rate of erythrocyte hemolysis. Compound **1** proved to show an effective antioxidative activity. Additionaly, compounds **4**, **5** and **8a** showed good activities as well. Meanwhile, compounds **2a**, **2b**, **2d**, **2e**, **3** and **6** showed a moderate activity toward the ABTS method. Compounds

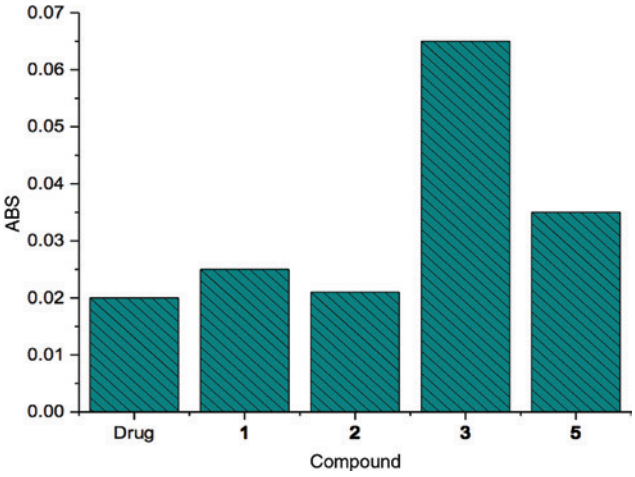


Figure 3: Bleomycin-dependent DNA damage.

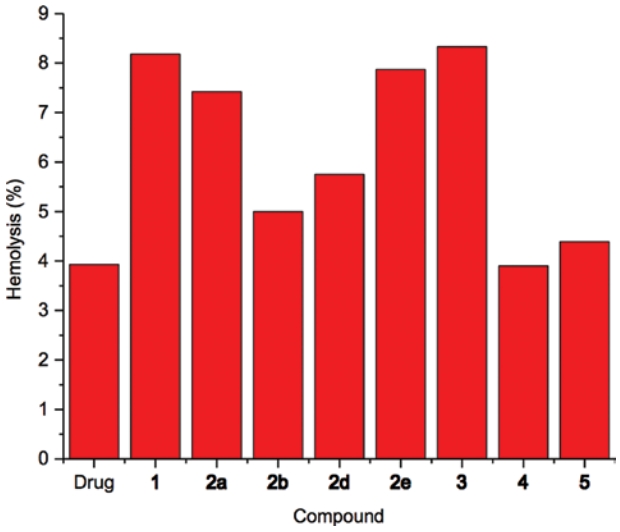


Figure 1: Erythrocyte hemolysis (%).

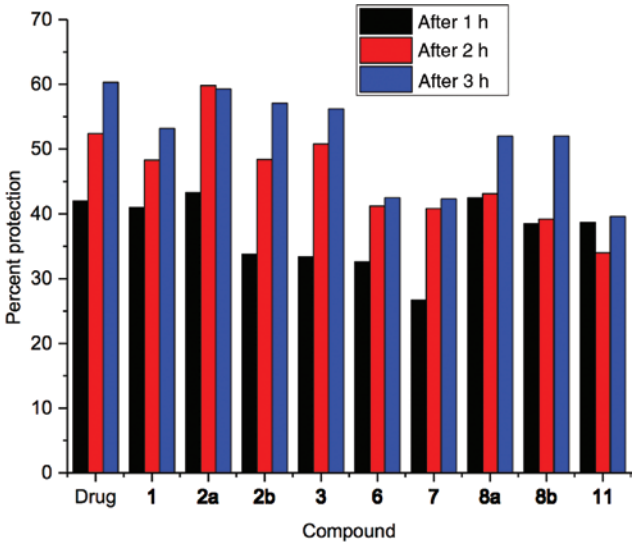


Figure 4: Anti-inflammatory activity.

2b, **4** and **5** exhibited potent activities toward erythrocyte hemolysis with ascorbic acid (Figures 1 and 2).

3.3.2 Bleomycin-dependent DNA damage

Bleomycins are routinely used as antitumor agents because they are a family of antitumors. Some of the synthesized compounds have been examined. The results indicated that compounds **1** and **2a** showed potent protective activity to DNA. Among the tested compounds, compound **5** showed good protection activity against DNA damage (Figure 3).

3.3.3 Anti-inflammatory evaluation

Carrageenan-induced paw edema test in rats was used to evaluate the tested compounds' activities. The results suggested that all the test compounds protected rats from carrageenan-induced inflammation. On the one hand, compounds **2a** and **b** showed higher activities than diclofenac sodium. On the other hand, compounds **1** and **3** were equipotent to diclofenac sodium (Figure 4).

4 Conclusion

In conclusion, the objective of the present work was the synthesis of new fused heterocyclic compounds with biological evaluation through a simple, environmentally friendly, and useful method (microwave synthesis). Moreover, some computational approaches have been achieved to rationalize experiments by focusing on compounds that are more likely to possess the desired activity and bioavailability.

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Conflict of interest statement: The authors declare to have no conflicts of interest.

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