Research Article

Synthesis, Single Crystal X-Ray Structure, and Antimicrobial Activity of 6-(1,3-Benzodioxol-5-ylmethyl)-5-ethyl-2- {[2-(morpholin-4-yl)ethyl]sulfanyl}pyrimidin-4(3H)-one

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Synthesis, X-ray structure and antimicrobial activity of 6-(1,3-benzodioxol-5-ylmethyl)-5-ethyl-2-
{[2-(morpholin-4-yl)ethyl]sulfanyl}pyrimidin-4(3H)-one (**8**) are reported. Compound **8** exhibited activity towards *S. aureus* with MIC value of 0.0619 $\mu\text{mol/mL}$ while it showed activity towards *B. subtilis*, *B. cereus*, *C. albicans*, and *A. niger* with MIC = 0.1859 $\mu\text{mol/mL}$. Single crystal X-ray structure of the title compound **8** confirmed its S-alkylation. The title compound crystallizes in the triclinic, *P*-1, $a = 11.1220$ (5) Å, $b = 12.2241$ (5) Å, $c = 21.5246$ (9) Å, $\alpha = 88.958$ (2)°, $\beta = 79.836$ (2)°, $\gamma = 79.384$ (2)°, $V = 2830.9$ (2) Å³, $Z = 6$, $R(F) = 0.046$, $wR(F^2) = 0.110$, $T = 100$ K. The crystal structure is stabilized by weak intermolecular C–H $\cdots$ O and N–H $\cdots$ O hydrogen interactions.

1. Introduction

Pyrimidines are attractive scaffolds for drug design and development since their discovery as components of DNA and RNA. Besides, pyrimidines are incorporated in a plethora of bioactive molecules with various biological activities. The chemotherapeutic effect of pyrimidines could be attributed to their ability to inhibit vital enzymes responsible for the nucleic acid biosynthesis. Several pyrimidines showed antiviral [1–3], antimicrobial [4–8], anticancer [9–11], anti-inflammatory [12, 13], antileishmanial [14, 15], and cardiovascular activities [16, 17].

On the other hand, a sizable number of bioactive molecules bearing 1,3-benzodioxole moiety exhibited multiple biological activities like antioxidant [18, 19], anticancer

[20, 21], anti-inflammatory [22, 23], anticonvulsant [24], antihypertensive [25], antidepressant [26], immunomodulators [27], and antiprotozoal [28, 29].

In continuation of our ongoing research on the synthesis of potential antimicrobial pyrimidinones [30], we report herein the synthesis, single crystal X-ray structure, and antimicrobial activity of new pyrimidinone derivative incorporating 1,3-benzodioxole moiety, namely, 6-(1,3-benzodioxol-5-ylmethyl)-5-ethyl-2-
{[2-(morpholin-4-yl)ethyl]sulfanyl}pyrimidin-4(3H)-one.

2. Experimental

2.1. General. Melting points were determined on a Galenkamp melting point apparatus and are uncorrected. Silica

gel TLC (thin layer chromatography) plates from Merck (silica gel precoated aluminium plates with fluorescent indicator at 254 nm) were used for thin layer chromatography. Visualization was performed by illumination with UV light source (254 nm). Compound **4** was prepared according to the reported literature procedures [31–33]. The tested microorganisms (Gram-positive bacteria, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* NRRL 4219, and *Bacillus cereus*; pathogenic fungi, *Candida albicans* ATCC 10231 and *Aspergillus niger* NRRL 599) were obtained from MIRCIN Cairo, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The reference drugs ampicillin trihydrate (CAS 7177-48-2) and clotrimazole (CAS 23593-75-1) were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). The X-ray diffraction measurements of compound **8** were performed using Bruker Venture D8 diffractometer.

2.2. Synthesis of 6-(1,3-Benzodioxol-5-ylmethyl)-5-ethyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (7). A suspension of activated zinc dust [30] (7 g) in dry THF (30 mL) was heated to reflux. Few drops of ethyl 2-bromobutanoate (**5**) were added and the reaction mixture was refluxed for 10 min. The piperonylcarbonitrile (**4**) (2.42 g, 0.015 mol) was added in one portion while the rest of ester **5** (5.85 g, 0.03 mol) was added dropwise. After complete addition, the reaction mixture was refluxed for 30 min, then diluted with THF (100 mL), and quenched by addition of sat. aq. K_2CO_3 solution (30 mL). The reaction mixture was stirred for 1 h at room temperature and then the THF layer was removed and the residue was washed with THF (3 $\times$ 20 mL). The combined THF fractions were stirred with 10% aq. HCl (20 mL) for 30 min. The solution was concentrated under reduced pressure and diluted with CH_2Cl_2 (100 mL). The organic phase was washed with sat. aq. $NaHCO_3$ solution (2 $\times$ 50 mL), dried (Na_2SO_4), and evaporated under reduced pressure to give the respective β -ketoester **6** as a pale yellow viscous oil. The crude β -ketoester **6** (2.78 g, 0.01 mol) was added to a boiling solution of sodium ethoxide (prepared from 4.9 g of Na in 100 mL of absolute EtOH) containing thiourea (11.42 g, 0.15 mol). The reaction mixture was heated to reflux for 24 h. The solvent was evaporated to dryness under reduced pressure and the residue was dissolved in H_2O (100 mL). The product was precipitated by addition of conc. HCl till pH = 4. The precipitate was filtered off, washed with H_2O , and dried to give the corresponding thiouracil derivative **7**. The crude **7** was purified by recrystallization (ethanol) to afford pure **7** in 46% yield as a white solid mp 183–185°C.

2.3. Synthesis of 6-(1,3-Benzodioxol-5-ylmethyl)-5-ethyl-2-[[2-(morpholin-4-yl)ethyl]sulfanyl]pyrimidin-4(3H)-one (8). Anhydrous potassium carbonate (0.304 g, 2.2 mmol) was added to a solution of thiouracil **7** (0.29 g, 1 mmol) in dry DMF (5 mL) followed by addition of *N*-(2-chloroethyl)morpholine hydrochloride (0.21 g, 1.1 mmol). The reaction mixture was stirred at room temperature for 24 h; then the solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel column with

$CHCl_3$ to afford the corresponding 2-[2-(morpholin-4-yl)ethyl]thiouracil derivative **8** in 58% yield as a white solid mp 138–140°C.

2.4. Crystal Structure Determination. Slow evaporation of the pure compound **8** in dimethyl sulfoxide yielded its colourless single crystals. A colourless block-shaped single crystal of suitable size, 0.65 $\times$ 0.32 $\times$ 0.19 mm, was selected for X-ray diffraction analysis. Data were collected on a Bruker Venture D8 CMOS area diffractometer equipped with graphite monochromatic MoK α radiation (λ = 0.71073 Å) at 100 K. Cell refinement and data reduction were done by Bruker SAINT [34]; program used to solve structure and refine structure is SHELXTL [35]. The final refinement was performed by full-matrix least-squares techniques with anisotropic thermal data for non hydrogen atoms on F^2 . All the hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. Multiscan absorption correction was applied by use of SADABS software [34]. The crystallographic data and refinement information are summarized in Table 1.

2.5. Antimicrobial Activity by the Agar Disc-Diffusion Method. Sterile nutrient, malt extract, and Czapek's dox agar media were inoculated, separately, with 100 μ L cell suspension of the chosen bacteria, *Candida albicans*, and *Aspergillus niger*, respectively, and poured into Petri dishes (20 cm diameter). The test compound **8** (200 μ g/10 mm diameter disc) was placed onto the surface of the agar Petri dishes. The antimicrobial potential was expressed as the diameter of the growth inhibition zone in mm [36].

2.6. Minimum Inhibitory Concentrations (MICs). The minimum inhibitory concentration (MIC) of the test compound **8** was evaluated using serial dilutions technique [37]. Different concentrations ranging from 50 to 200.0 μ g for the test compound **8** were dissolved in dimethyl sulfoxide (1 mL) and were placed on filter paper disc (1 cm diameter). The discs were deposited on the surface of inoculated agar plates and kept at low temperature before incubation which favours diffusion over microbial growth to detect the inhibition zone clearly. The plates were incubated at 30°C for 24 h for bacteria and *Candida albicans* and for 48 h for *Aspergillus niger*.

3. Results and Discussion

3.1. Chemistry. The commercially available piperonal (**1**) was elaborated to piperonylcarbonitrile (**4**) as portrayed in Scheme 1. Thus, piperonal (**1**) was reduced with sodium borohydride in methanol at room temperature to give the piperonyl alcohol (**2**) [31] in almost quantitative yield. The alcohol **2** was allowed to react with thionyl chloride to give the chloro derivative **3** [32]. Subsequently, compound **3** was subjected to nucleophilic substitution using sodium cyanide and a catalytic amount of potassium iodide in dimethylformamide to yield the respective piperonylcarbonitrile (**4**) [33] in about 75% overall yield.

Scheme 2 illustrates the synthetic pathway to achieve the target compound **8** and its intermediates. Compound **4**

SCHEME 1: Synthesis of the intermediate piperonylcarbonitrile (4). Reagents and conditions: (i) NaBH_4 , methanol, RT, 18 h; (ii) SOCl_2 , RT, 18 h; (iii) NaCN , KI , DMF , RT, 18 h.

SCHEME 2: Synthesis of the target compound 8. Reagents and conditions: (i) (1) Zn/THF , (2) K_2CO_3 , and (3) HCl ; (ii) (1) NH_2CSNH_2 , NaOEt , ethanol, reflux, 24 h and (2) HCl ; (iii) N -(2-chloroethyl) morpholine hydrochloride, K_2CO_3 , DMF , RT, 18 h.

was allowed to react with ethyl 2-bromobutanoate (5) in dry tetrahydrofuran in the presence of zinc dust under Reformatsky conditions [38] to furnish ethyl 4-(1,3-benzodioxol-5-yl)-2-ethyl-3-oxobutanoate (6) in good yield. Reaction of thiourea with the crude β -ketoester 6 in absolute ethanol in the presence of sodium ethoxide gave the pivotal 6-(1,3-benzodioxol-5-ylmethyl)-5-ethyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (7) in 46% yield. Thiouracil 7 was reacted with N -(2-chloroethyl) morpholine hydrochloride in the presence of anhydrous potassium carbonate in dimethylformamide at room temperature to afford the title compound 6-(1,3-benzodioxol-5-ylmethyl)-5-ethyl-2-([2-(morpholin-4-yl)ethyl]sulfanyl) pyrimidin-4(3*H*)-one (8) in 58% yield. Single crystal X-ray crystallography confirmed the 3D structure of compound 8.

3.2. Crystal Structure of Compound 8. Single crystal X-ray crystallography is a doubtlessly decisive analytical tool which can confirm the 3D structure of the target compound 8, particularly its *S*-alkylation. Fortunately, we have succeeded to get single crystals of compound 8 which were suitable for X-ray crystallography (Figure 1).

The asymmetric unit contains three molecules of the title compound 8. The molecular packing in the crystal structure is stabilized by the weak intermolecular interactions (Figure 2, Table 2), of which O3B, O2A, and O4B work as acceptor while N2B, C3A, and C19B work as donors.

The distance $\text{H1NB}\cdots\text{O3B}$ is 1.87 (2) Å, $\text{H3AB}\cdots\text{O2A}$ is 2.5300, and $\text{H19D}\cdots\text{O4B}$ is 2.5600 Å, and the angles between $\text{N2B-H1NB}\cdots\text{O3B}$, $\text{C3A-H3AB}\cdots\text{O2A}$, and $\text{C19B-H19D}\cdots\text{O4B}$ are 173 (2), 146.00 (2), and 140.00° (2), respectively. There are four intramolecular H-bonds in the molecules: in the first molecule, there is one H-bond between $\text{C16A-H16B}\cdots\text{N1A}$; in the second molecule, there is one H-bond between $\text{C15B-H15C}\cdots\text{N1B}$; and in the third molecule, there are two H-bonds between $\text{C16C-H16E}\cdots\text{N1C}$ and $\text{C17C-H17F}\cdots\text{O1C}$. The selected bond lengths, bond angles, and bond torsion angles are listed in Table 3.

3.3. In Vitro Antimicrobial Activity. The *in vitro* antimicrobial activity of compound 8 was evaluated against Gram-positive bacteria (*S. aureus*, *B. subtilis*, and *B. cereus*) and pathogenic fungi (*C. albicans* and *Aspergillus niger*). The obtained data, expressed as diameter of the inhibition zone (DIZ) and minimum inhibitory concentration (MIC) for the test compound 8 as well as for the reference antibacterial standard, ampicillin, and the reference antifungal standard, clotrimazole, are shown in Tables 4 and 5.

The preliminary antimicrobial potential of the test compound 8 was evaluated using DIZ assay and the results are given in Table 4. Compound 8 exhibited good antimicrobial activity with DIZ values in the range of 17–20 mm against the test microorganisms. Compound 8 showed no activity

FIGURE 1: ORTEP diagram of the title compound 8 drawn at 50% ellipsoids for non hydrogen atoms.

FIGURE 2: Crystal packing showing intermolecular C-H...O hydrogen bonds as dashed lines.

TABLE 1: Crystallographic data and refinement information.

Empirical formula	C ₂₀ H ₂₅ N ₃ O ₄ S
Formula weight	403.49
Temperature (K)	100
Crystal system	Triclinic
Space group	<i>P</i> -1
Mo <i>K</i> α radiation, λ	0.71073 Å
<i>a</i> (Å)	11.1220 (5)
<i>b</i> (Å)	12.2241 (5)
<i>c</i> (Å)	21.5246 (9)
α (°)	88.958 (2)
β (°)	79.836 (2)
γ (°)	79.384 (2)
<i>V</i> (Å ³)	2830.9 (2)
<i>Z</i>	6
<i>F</i> (000)	1284
θ range (°)	2.3–28.0
μ (mm ^{−1})	0.21
Density (calc.) (g/cm ³)	1.420
Crystal shape and color	Block, colorless
Crystal size (mm ³)	0.65 × 0.32 × 0.19
<i>h</i> / <i>k</i> / <i>l</i>	−14,14/−16,16/−28,28
Measured reflections	86637
Independent reflections	14111 [<i>R</i> _{int} = 0.071]
Reflections with <i>I</i> > 2σ(<i>I</i>)	9629
Goodness-of-fit on <i>F</i> ²	1.01
<i>R</i> [<i>F</i> ² > 2σ(<i>F</i> ²)]	0.046
<i>wR</i> (<i>F</i> ²)	0.110
Δρ _{max} (e Å ^{−3})	0.37
Δρ _{min} (e Å ^{−3})	−0.33

TABLE 2: Hydrogen-bond geometry (Å, °).

D–H...A	D–H	H...A	D...A	D–H...A
N2C–H1NC...O3A	0.92 (2)	1.85 (2)	2.7721 (18)	178.0 (12)
N2B–H1NB...O3B ⁱ	0.92 (2)	1.87 (2)	2.7904 (19)	173 (2)
N2A–H1NA...O3C	0.97 (2)	1.76 (2)	2.7330 (18)	179 (3)
C3A–H3AB...O2A ⁱⁱ	0.9700	2.5300	3.371 (2)	146.00
C15B–H15C...N1B	0.9700	2.4800	2.882 (2)	105.00
C16A–H16B...N1A	0.9700	2.6100	3.198 (2)	119.00
C16C–H16E...N1C	0.9700	2.5800	3.170 (2)	120.00
C17C–H17F...O1C	0.9700	2.5900	3.441 (2)	146.00
C19A–H19B...O2B	0.9700	2.5000	3.462 (2)	172.00
C19B–H19D...O4B ⁱⁱⁱ	0.9700	2.5600	3.366 (3)	140.00

Symmetry codes: (i) $-x + 1, -y + 3, -z$; (ii) $-x, -y + 1, -z + 1$; (iii) $-x + 1, -y + 3, -z + 1$.

against Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*.

Minimum inhibitory concentration assay was performed in order to get more insight into the antimicrobial activity of

compound **8** as a new antimicrobial pyrimidinone derivative. Compound **8** displayed antimicrobial activity towards the tested bacteria and pathogenic fungi with MIC values in the range of 0.0619–0.1859 μmol/mL (Table 5).

TABLE 3: Selected geometric parameters (Å, °) for one molecule.

S1A–C14A	1.7546 (18)	O3C–C13C	1.244 (2)
S1A–C15A	1.8062 (18)	O4C–C18C	1.431 (2)
O1A–C2A	1.377 (2)	N2A–C13A	1.380 (2)
O1A–C3A	1.421 (3)	N3A–C17A	1.463 (2)
O2A–C4A	1.383 (2)	N3A–C20A	1.469 (2)
O2A–C3A	1.432 (3)	N3A–C16A	1.466 (2)
O3A–C13A	1.241 (2)	N1B–C9B	1.380 (2)
O4A–C18A	1.425 (2)	N1B–C14B	1.295 (2)
O4A–C19A	1.425 (2)	N2B–C14B	1.365 (2)
C14A–S1A–C15A	101.81 (8)	N3A–C17A–C18A	110.66 (17)
C2A–O1A–C3A	105.29 (15)	N3A–C20A–C19A	109.97 (14)
C3A–O2A–C4A	104.85 (15)	O1B–C2B–C1B	128.33 (17)
C18A–O4A–C19A	109.78 (13)	O1B–C2B–C4B	109.39 (15)
C9A–N1A–C14A	116.61 (14)	O3B–C13B–N2B	119.86 (15)
C13A–N2A–C14A	122.13 (14)	N2B–C13B–C10B	115.59 (15)
C16A–N3A–C17A	108.97 (14)	S1B–C14B–N1B	121.81 (13)
C17A–N3A–C20A	108.05 (13)	N1B–C14B–N2B	124.00 (16)
C16A–N3A–C20A	110.73 (13)	S1B–C14B–N2B	114.19 (12)
O1A–C2A–C4A	110.00 (15)	O2C–C4C–C2C	109.89 (15)
O1A–C2A–C1A	127.51 (18)	N1C–C9C–C8C	112.44 (14)
O1A–C3A–O2A	108.23 (15)	N1C–C9C–C10C	123.70 (15)
O2A–C4A–C2A	109.69 (16)	O3C–C13C–C10C	124.24 (16)
O2A–C4A–C5A	128.57 (18)	O3C–C13C–N2C	119.71 (15)
N1A–C9A–C10A	123.90 (15)	N2C–C13C–C10C	116.05 (15)
N1A–C9A–C8A	112.67 (14)	S1C–C14C–N2C	113.52 (12)
O3A–C13A–N2A	119.66 (15)	S1C–C14C–N1C	122.83 (13)
N2A–C13A–C10A	115.36 (15)	N1C–C14C–N2C	123.65 (16)
O3A–C13A–C10A	124.97 (16)	S1C–C15C–C16C	112.16 (13)
S1A–C14A–N1A	122.83 (13)	N3C–C16C–C15C	113.16 (15)
S1A–C14A–N2A	113.20 (12)	N3C–C17C–C18C	111.34 (16)
N1A–C14A–N2A	123.97 (16)	O4C–C18C–C17C	111.12 (14)
S1A–C15A–C16A	111.98 (13)	O4C–C19C–C20C	111.83 (16)
N3A–C16A–C15A	112.40 (15)	N3C–C20C–C19C	110.99 (14)

TABLE 4: Diameter of growth inhibition zone (mm) of the target compound **8**, ampicillin, and clotrimazole towards the test microorganisms.

Compound number	Diameter of growth inhibition zone $\pm$ SD (mm)*				
	Gram-positive bacteria			Pathogenic fungi	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
8	20 $\pm$ 0.50	18 $\pm$ 1.00	19 $\pm$ 1.50	17 $\pm$ 1.50	20 $\pm$ 1.50
Ampicillin	32 $\pm$ 1.00	28 $\pm$ 1.00	27 $\pm$ 0.50		
Clotrimazole				35 $\pm$ 1.00	32 $\pm$ 0.00

*The arithmetic mean of the inhibition zone diameters in mean $\pm$ standard deviation in mm.

TABLE 5: The minimum inhibitory concentration (MIC, μ mol/mL) values for the target compound **8**, ampicillin, and clotrimazole towards the test microorganisms.

Compound number	The minimum inhibitory concentrations (MICs, μ mol/mL)*				
	Gram-positive bacteria			Pathogenic fungi	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
8	0.0619	0.1859	0.1859	0.1859	0.1859
Ampicillin	0.0258	0.0258	0.0258		
Clotrimazole				0.0261	0.0348

*The lowest concentration of the test compound that inhibits the growth of microorganism (μ mol/mL).

4. Conclusions

6-(1,3-Benzodioxol-5-ylmethyl)-5-ethyl-2-[[2-(morpholin-4-yl)ethyl]sulfanyl]pyrimidin-4(3H)-one (**8**) was synthesized as a novel antimicrobial pyrimidinone derivative bearing 1,3-benzodioxole moiety. The 3D structure of the target compound **8** and its S-alkylation were confirmed *via* single crystal X-ray crystallography. The antimicrobial evaluation of the title compound **8** revealed its dual antibacterial (MIC value = 0.0619 towards *S. aureus*) and antifungal activities (MIC value = 0.1859 towards both *C. albicans* and *A. niger*).

Additional Materials

Crystallographic data of compound **8** have been deposited with the Cambridge Crystallographic Data Center (supplementary publication number CCDC-991136). Copies of the data may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (deposit@ccdc.cam.ac.uk).

Conflict of Interests

The authors have declared that there is no conflict of interests.

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References

- [1] E. El-Bendary and F. Badria, "Synthesis, DNA-binding, and antiviral activity of certain pyrazolo[3, 4-*d*]pyrimidine derivatives," *Archiv der Pharmazie*, vol. 333, no. 4, pp. 99–103, 2000.
- [2] J.-H. Chern, K.-S. Shia, T.-A. Hsu et al., "Design, synthesis, and structure-activity relationships of pyrazolo[3,4-*d*]pyrimidines: a novel class of potent enterovirus inhibitors," *Bioorganic & Medicinal Chemistry Letters*, vol. 14, no. 10, pp. 2519–2525, 2004.
- [3] M. S. Novikov, V. T. Valuev-Elliston, D. A. Babkov et al., "N¹, N³-disubstituted uracils as nonnucleoside inhibitors of HIV-1 reverse transcriptase," *Bioorganic & Medicinal Chemistry Letters*, vol. 21, no. 5, pp. 1150–1158, 2013.
- [4] A. Ali, G. E. Taylor, K. Ellsworth et al., "Novel pyrazolo[3,4-*d*]pyrimidine-based inhibitors of *Staphylococcus aureus* DNA polymerase III: design, synthesis, and biological evaluation," *Journal of Medicinal Chemistry*, vol. 46, no. 10, pp. 1824–1830, 2003.
- [5] A. Trivedi, D. Dodiya, J. Surani et al., "Facile one-pot synthesis and antimycobacterial evaluation of pyrazolo[3,4-*d*]pyrimidines," *Archiv der Pharmazie*, vol. 341, no. 7, pp. 435–439, 2008.
- [6] M. Bakavoli, G. Bagherzadeh, M. Vaseghifar et al., "Molecular iodine promoted synthesis of new pyrazolo[3,4-*d*]pyrimidine derivatives as potential antibacterial agents," *European Journal of Medicinal Chemistry*, vol. 45, no. 2, pp. 647–650, 2010.
- [7] D. Ranganath, A. Mazumdar, S. Mulukuri, R. Doonaboina, M. Devarakonda, and M. R. Prasad, "A novel and eco-friendly procedure for the synthesis of some pyrazolo-thiadiazolo-pyrimidinones and its *in vitro* anti-bacterial activity," *Medicinal Chemistry Research*, vol. 21, no. 9, pp. 2458–2464, 2012.
- [8] O. A. Al-Deeb, A. A. Al-Turkistani, E. S. Al-Abdullah, N. R. El-Brollosy, E. E. Habib, and A. A. El-Emam, "Pyrimidine-5-carbonitriles-part III: synthesis and antimicrobial activity of novel 6-(2-substituted propyl)-2, 4-disubstituted pyrimidine-5-carbonitriles," *Heterocyclic Communications*, vol. 19, no. 6, pp. 411–419, 2013.
- [9] A. Angelucci, S. Schenone, G. L. Gravina et al., "Pyrazolo[3,4-*d*]pyrimidines c-Src inhibitors reduce epidermal growth factor-induced migration in prostate cancer cells," *European Journal of Cancer*, vol. 42, no. 16, pp. 2838–2845, 2006.
- [10] F. Manetti, A. Santucci, G. A. Locatelli et al., "Identification of a novel pyrazolo[3,4-*d*]pyrimidine able to inhibit cell proliferation of a human osteogenic sarcoma *in vitro* and in a xenograft model in mice," *Journal of Medicinal Chemistry*, vol. 50, no. 23, pp. 5579–5588, 2007.
- [11] A. Lauria, I. Abbate, C. Patella, A. Martorana, G. Dattolo, and A. M. Almerico, "New annelated thieno[2, 3-*e*][1, 2, 3]triazolo[1, 5-*a*]pyrimidines, with potent anticancer activity, designed through VLAK protocol," *European Journal of Medicinal Chemistry*, vol. 62, pp. 416–424, 2013.
- [12] M. Amir, S. Javed, and H. Kumar, "Pyrimidine as antiinflammatory agent: a review," *Indian Journal of Pharmaceutical Sciences*, vol. 69, no. 3, pp. 337–343, 2007.
- [13] A. P. Keche, G. D. Hatnapure, R. H. Tale, A. H. Rodge, S. S. Birajdar, and V. M. Kamble, "A novel pyrimidine derivatives with aryl urea, thiourea and sulfonamide moieties: synthesis, anti-inflammatory and antimicrobial evaluation," *Bioorganic & Medicinal Chemistry Letters*, vol. 22, no. 10, pp. 3445–3448, 2012.
- [14] D. J. Nelson, S. W. LaFon, J. V. Tuttle et al., "Allopurinol ribonucleoside as an antileishmanial agent. Biological effects, metabolism, and enzymatic phosphorylation," *The Journal of Biological Chemistry*, vol. 254, no. 22, pp. 11544–11549, 1979.
- [15] J. J. Marr, R. L. Berens, D. J. Nelson et al., "Antileishmanial action of 4-thiopyrazolo(3,4-*d*)pyrimidine and its ribonucleoside. Biological effects and metabolism," *Biochemical Pharmacology*, vol. 31, no. 2, pp. 143–148, 1982.
- [16] S. Guccione, M. Modica, J. Longmore et al., "Synthesis and NK-2 antagonist effect of 1, 6-diphenyl-pyrazolo[3, 4-*d*]thiazolo[3, 2-*a*]-4H-pyrimidin-4-one," *Bioorganic & Medicinal Chemistry Letters*, vol. 6, no. 1, pp. 59–64, 1996.
- [17] H. S. Ahn, A. Bercovici, G. Boykow et al., "Potent tetracyclic guanine inhibitors of PDE1 and PDE5 cyclic guanosine monophosphate phosphodiesterases with oral antihypertensive activity," *Journal of Medicinal Chemistry*, vol. 40, no. 14, pp. 2196–2210, 1997.
- [18] M. Himaja, K. Vandana, A. Ranjitha, M. Ramana, and A. Karigar, "Synthesis, docking studies and antioxidant activity of 1, 3-benzodioxole-5-carboxyl amino acids and dipeptides," *International Research Journal of Pharmacy*, vol. 2, no. 6, pp. 57–61, 2011.
- [19] R. Joshi, M. S. Kumar, K. Satyamoorthy, M. K. Unnikrisnan, and T. Mukherjee, "Free radical reactions and antioxidant activities of sesamol: pulse radiolytic and biochemical studies," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 7, pp. 2696–2703, 2005.

- [20] N. Micale, M. Zappalà, and S. Grasso, "Synthesis and cytotoxic activity of 1,3-benzodioxole derivatives. Note II," *Il Farmaco*, vol. 58, no. 5, pp. 351–355, 2003.
- [21] A. C. L. Leite, K. P. da Silva, I. A. de Souza, J. M. de Araújo, and D. J. Brondani, "Synthesis, antitumour and antimicrobial activities of new peptidyl derivatives containing the 1,3-benzodioxole system," *European Journal of Medicinal Chemistry*, vol. 39, no. 12, pp. 1059–1065, 2004.
- [22] M. H. Mehmood and A. H. Gilani, "Pharmacological basis for the medicinal use of black pepper and piperine in gastrointestinal disorders," *Journal of Medicinal Food*, vol. 13, no. 5, pp. 1086–1096, 2010.
- [23] Y. P. Hwang, H. J. Yun, H. G. Kim et al., "Suppression of phorbol-12-myristate-13-acetate-induced tumor cell invasion by piperine via the inhibition of PKC α /ERK1/2-dependent matrix metalloproteinase-9 expression," *Toxicology Letters*, vol. 203, no. 1, pp. 9–19, 2011.
- [24] M. N. Aboul-Enein, A. A. El-Azzouny, M. I. Attia et al., "Design and synthesis of novel stiripentol analogues as potential anticonvulsants," *European Journal of Medicinal Chemistry*, vol. 47, no. 1, pp. 360–369, 2012.
- [25] V. Diwan, H. Poudyal, and L. Brown, "Piperine attenuates cardiovascular, liver and metabolic changes in high carbohydrate, high fat-fed rats," *Cell Biochemistry and Biophysics*, vol. 67, no. 2, pp. 297–304, 2013.
- [26] S. Li, C. Wang, M. Wang, W. Li, K. Matsumoto, and Y. Tang, "Antidepressant like effects of piperine in chronic mild stress treated mice and its possible mechanisms," *Life Sciences*, vol. 80, no. 15, pp. 1373–1381, 2007.
- [27] D. P. Bezerra, F. O. de Castro, A. P. N. Alves et al., "In vitro and in vivo antitumor effect of 5-FU combined with piplartine and piperine," *Journal of Applied Toxicology*, vol. 28, no. 2, pp. 156–163, 2008.
- [28] N. Flores, I. A. Jiménez, A. Giménez et al., "Antiparasitic activity of prenylated benzoic acid derivatives from *Piper* species," *Phytochemistry*, vol. 70, no. 5, pp. 621–627, 2009.
- [29] F. Cotinguiba, L. O. Regasini, V. da Silva Bolzani et al., "Piperamides and their derivatives as potential anti-trypansomal agents," *Medicinal Chemistry Research*, vol. 18, no. 9, pp. 703–711, 2009.
- [30] M. I. Attia, A. A. El-Emam, A. A. Al-Turkistani, A. L. Kansoh, and N. R. El-Brollosy, "Synthesis of novel 2-(substituted amino)alkylthiopyrimidin-4(3H)-ones as potential antimicrobial agents," *Molecules*, vol. 19, no. 1, pp. 279–290, 2014.
- [31] L. Feng, K. Lv, M. Liu et al., "Synthesis and in vitro antibacterial activity of gemifloxacin derivatives containing a substituted benzyloxime moiety," *European Journal of Medicinal Chemistry*, vol. 55, pp. 125–136, 2012.
- [32] W. Cunico, C. R. Gomes, M. Moreth et al., "Synthesis and antimalarial activity of hydroxyethylpiperazine derivatives," *European Journal of Medicinal Chemistry*, vol. 44, no. 3, pp. 1363–1368, 2009.
- [33] G. Blay, L. Cardona, B. García, L. Lahoz, and J. Pedro, "The reduction of α , β -unsaturated nitriles and α -halonitriles with sodium hydrogen telluride," *Tetrahedron*, vol. 52, no. 25, pp. 8611–8618, 1996.
- [34] Bruker, APEX2, SAINT and SADABS, Bruker AXS, Madison, Wis, USA, 2009.
- [35] G. M. Sheldrick, "A short history of SHELX," *Acta Crystallographica A: Foundations of Crystallography*, vol. 64, no. 1, pp. 112–122, 2008.
- [36] C. Penna, S. Marino, G. Gutkind, M. Clavin, G. Ferraro, and V. Martino, "Antimicrobial activity of *Eupatorium* species growing in Argentina," *Journal of Herbs, Spices & Medicinal Plants*, vol. 5, no. 2, pp. 21–28, 1998.
- [37] T. D. Wikins, L. V. Holdeman, I. J. Abramson, and W. Moore, "Standardized single-disc method for antibiotic susceptibility testing of anaerobic bacteria," *Antimicrobial Agents and Chemotherapy*, vol. 1, no. 6, pp. 451–459, 1972.
- [38] A. Fürstner, "Recent advancements in the Reformatsky reaction," *Synthesis*, no. 8, pp. 571–590, 1989.