ONE-POT SYNTHESIS, ANTIMICROBIAL SCREENING AND ADME STUDY OF BENZOPYRONE[8,7-E][1,3]OXAZINES

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Abstract:

Mannich-type condensation-cyclization reaction of 7-Hydroxy-4-methyl benzopyrones with formaldehyde and primary amines in toluene at reflux, benzopyrone[8,7-e][1,3]oxazine derivatives were created and confirmed by spectral analyses. Antimicrobial screening showed promising results against various bacterial & fungal strains. ADME (Adsorption Distribution Metabolism Excretion) assessment of these drugs was performed. Title compounds passed ADME evaluation and showed good absorption of drugs in cells.

Keywords: Benzoxazine, Antimicrobial screening, ADME studies.

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1. Introduction

Benzoxazine possesses Numerous antibiotics [1], antirheumatic medicines [2], calcium channel antagonists [3], pharmaceuticals for the central nervous system [4], and analgesics [1-5]. In vascular smooth muscle, several 3,4-dihydro-2H-1,4-benzoxazine derivatives have been found to open potassium channels (PCOs) [6]. Numerous benzoxazines have been found to have intriguing anti-Mycobacterium tuberculosis activity [7]. Additionally, some synthetic methotrexate derivatives, including the benzoxazine moiety, were discovered to be effective and secure candidates for antirheumatic medications [8]. Other heterocyclic structures of biological significance have also been synthesized using benzoxazine derivatives as intermediates [9]. There have also been reports of several coumarins [8,7-e][1,3]oxazine derivatives having anti-inflammatory and anticancer properties [10-14]. Particularly for the treatment of Parkinson's disease, naphthoxazine derivatives have shown therapeutic promise [15-16]. Benzoxazine derivatives, in particular those with a 1,3-position, have been found to have potential antimicrobial, antifungal, antiproliferative, and antibacterial activities [17-23]. Also described as strong non-steroidal progesterone receptor agonists are 6-arylbenzoxazines [24]. Several 1,4-benzothiazine and 1,4-benzoxazine imidazole derivatives were synthesized and tested for their antifungal efficacy against a mouse experimental model of candidiasis, however, they frequently lacked in vitro action [25-28]. Looking toward the medicinal importance of benzoxazine derivatives, we decided to synthesize them and explore their antimicrobial activity. ADME study (absorption, distribution, metabolism, and excretion) was carried out to evaluate the biological compatibility of synthesized benzoxazine analogs.

Experimental

Materials and Methods: All the chemicals and solvents are indented from Sigma Aldrich. The melting points of the produced compounds were measured in the glass capillary method and uncorrected. IR spectra were captured on the Bruker IR Spectrometer. Elemental analysis values were within 0.4% of theoretical values. Using deuterated dimethyl sulfoxide (DMSO) and deuterated chloroform ($CDCl_3$) as solvents and tetramethyl silane as an internal standard ^{1}H NMR was obtained using a Bruker TD-65536 NMR (400MHz).

Synthesis of 7-hydroxy-4-methyl-2H-chromene-2-one **(1)**:

Pechmann condensation [29] of resorcinol and ethyl acetoacetate to yield 7-hydroxy-4-methyl-chromen-2-one.

General procedure of Benzopyrone[8,7-e][1,3]oxazine **(3a–3e)**:

Charged Formalin solution (2.57 mL, 35%, 33mmol), primary amine **2a – 2f** (15mmol) and toluene (15mL) in a 150 mL-3N-RBF. Stir the RM at 30-35℃ for 1 Hr. 7-hydroxy-4-methylbenzopyron-2[H]-one (2.91 gm, 15 mmol) (Lit [29]) in above reaction mixture. The reaction mixture refluxed for 4 Hr. Reaction completion was checked by TLC. The reaction mixture allows to cool at 30-35℃ and dried with anhydrous sodium sulphate. RM was filtered and distilled under vacuum till dryness. Obtained crude crystallized from ethanol to yield title compounds **3a–3f**.

4-methyl-9-phenyl-9,10-dihydro-2H,8H-chromeno[8,7- e][1,3]oxazin-2-one (**3a**)

Yield: 70%, m.p. 135℃. IR (KBr) cm^{-1}: 1731.12, 1433.40, 1356.60, 1314.12, 1265.79, 1215.25, 1057.73, 980.65. ^{1}H NMR (400MHz, DMSO-d_6) δ, ppm: 7.25 (1H, d, C(5)H); 7.15 (2H, d, (C(3)H -C_6H_5 , C(5)H -C_6H_5); 7.10 (2H, d, (C(2)H -C_6H_5 , C(6)H -C_6H_4); 6.90 (1H, t, C(4)H -C_6H_4); 6.76 (1H, d, C(6)H); 6.11 (1H, s, -C(CH_3)=CH-COO-); 6.99 (2H, s, –O-CH_2-

N–); 4.47 (2H, s, Ar-CH_2-N-); 2.43 (3H, s, Ar-C(CH_3)=CH-). EI-MS Mass calculated for $C_{18}H_{15}NO_5$ (m/z): 293.31, found $[M]^+$: 293.2. Elemental analysis Found: C, 73.70; H, 5.01; N, 4.75; O, 16.54.

4-methyl-9-(3-nitrophenyl)-9,10-dihydro-2H,8H-chromeno[8,7-e][1,3]oxazin-2-one (**3b**)

Yield: 78%, m.p. 110℃. IR (KBr) cm^{-1}: 2919.70, 2359.70, 1676.72, 1560.98, 1452.78, 1316.28, 1207.47, 1066.43, 980.73, 908.68. ^{1}H NMR (400MHz, DMSO-d_6) δ, ppm: 7.87 (2H, d, C(5)H, C(6)H -$C_6H_4NO_2$); 7.61 (2H, t, (C(2)H -$C_6H_4NO_2$, C(4)H -$C_6H_4NO_2$); 7.44 (1H, t, C(5)H -$C_6H_4NO_2$); 6.99 (2H, s, –O-CH_2-N–); 6.17 (1H, s, -C(CH_3)=CH-COO-); 7.04 (1H, d, C(6)H); 5.24 (2H, s, Ar-CH_2-N-); 2.42 (3H, s, Ar-C(CH_3)=CH-). EI-MS Mass calculated for $C_{18}H_{14}N_2O_5$ (m/z): 338.31, found $[M]^+$: 338.50. Elemental analysis found: C, 63.89; H, 4.10; N, 8.30; O, 23.71.

4-methyl-9-(4-nitrophenyl)-9,10-dihydro-2H,8H-chromeno[8,7-e][1,3]oxazin-2-one (**3c**)

Yield: 75%, m.p. 107℃. IR (KBr) cm^{-1}: 2919.70, 2359.70, 1676.72, 1513.26, 1390.41, 1316.26, 1207.47, 1136.60, 1066.43, 980.73. ^{1}H NMR (400 MHz, DMSO-d_6) δ, ppm: 8.12 (2H, d, (C(2)H -$C_6H_4NO_2$, C(4)H -$C_6H_4NO_2$); 7.72 (1H, d, C(5)H); 7.00 (3H, d, C(6)H, (C(3)H -$C_6H_4NO_2$, C(5)H -$C_6H_4NO_2$); 6.17 (1H, s, -C(CH_3)=CH-COO-); 6.01 (2H, s, –O-CH_2-N–); 4.82 (2H, s, Ar-CH_2-N-); 2.42 (3H, s, Ar-C(CH_3)=CH-). EI-MS Mass calculated for $C_{18}H_{14}N_2O_5$ (m/z): 338.31, found $[M]^+$: 338.13. Elemental analysis found: C, 63.92; H, 4.10; N, 8.25; O, 23.73.

4-(4-methyl-2-oxo-2H,8H-chromeno[8,7-e][1,3]oxazin-9(10H)-yl)benzenesulfonic acid (**3d**)

Yield: 60%, m.p. 125℃. IR (KBr) cm^{-1}: 2360.19, 1675.53, 1601.17, 1451.80, 1437.33, 1361.26, 1316.09, 1266.77, 1136.42, 1066.10, 980.95, 841.74. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.51 (1H, d, C(5)H); 7.26 (2H, d, C(2)H -$C_6H_4SO_3H$, C(6)H -$C_6H_4SO_3H$); 7.04 (2H, s, –O-CH_2-N–); 8.61 (1H, broad, s, SO_3H); 6.91 (3H, d, C(6)H, (C(3)H -$C_6H_4SO_3H$, C(5)H -$C_6H_4SO_3H$); 6.16 (1H, s, -C(CH_3)=CH-COO-); 4.38 (2H, s, Ar-CH_2-N-); 2.43 (3H, s, Ar-C(CH_3)=CH-). EI-MS Mass calculated for $C_{18}H_{15}NO_6S$ (m/z): 373.38, found $[M]^+$: 373.40. Elemental analysis found: C, 57.89; H, 4.01; N, 3.72; O, 25.69; S, 8.69.

4-methyl-9-(p-tolyl)-9,10-dihydro-2H,8H-chromeno[8,7-e][1,3]oxazin-2-one (**3e**)

Yield: 65%, m.p. 160℃. IR (KBr) cm^{-1}: 3023, 2883, 1713, 1596, 1512, 1493.1H NMR (400 MHz, CDCl3) δ, ppm: 7.36 (1H, d, C(5)H); 7.12 (3H, m, C(6)H, C(3)H -C_6H_4-Me, C(5)H -C_6H_4-Me); 6.77 (2H, d, C(2)H -C_6H_4-Me, C(6)H -C_6H_4-Me); 6.12 (1H, s, -C(CH_3)=CH-COO-); 5.41 (2H, s, –O-CH_2-N–); 4.79 (2H, s, Ar-CH_2-N-); 2.37 (3H, s, Ar-C(CH_3)=CH-); 2.26 (3H, s, -CH=C(CH_3)-CH=). EI-MS Mass calculated for $C_{19}H_{18}O_3N$ (m/z) 308.12 found $[M]^+$: 308.8. Elemental analysis found: C, 74.20; H, 5.50; N, 4.49; O, 15.81.

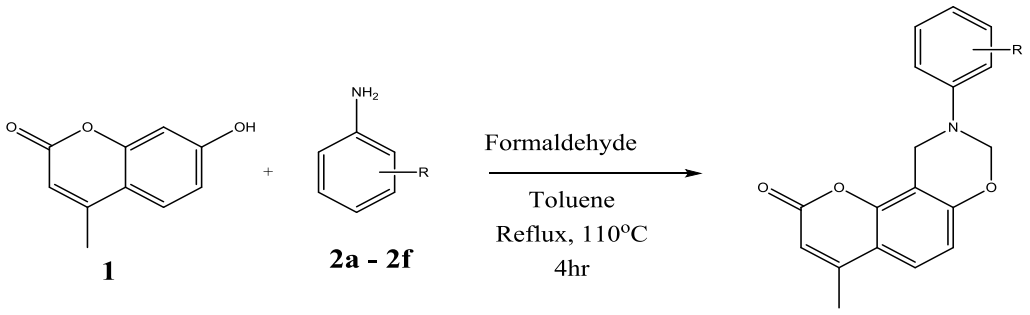
4-(4-methyl-2-oxo-2H,8H-chromeno[8,7-e][1,3]oxazin-9(10H)-yl)benzoicacid (**3f**)

Yield: 62%, m.p. 130℃. IR (KBr) cm^{-1}: 2929.94, 2360.02, 2339.20, 1698.61, 1681.08, 1651.14, 1604.14, 1558.11, 1541.49, 1454.43, 1360.57, 1136.70, 1066.23. ^{1}H NMR (400 MHz, $CDCl_3$) δ, ppm: 7.52 (3H, d, C(5)H, (C(2)H -C_6H_4-COOH, C(6)H -C_6H_4-COOH); 6.99 (2H, s, –O-CH_2-N–); 6.88 (3H, d, C(6)H, (C(3)H -C_6H_4-COOH, C(5)H -C_6H_4-COOH); 6.17 (1H, s, -C(CH_3)=CH-COO-); 4.47 (2H, s, Ar-CH_2-N-); 2.43 (3H, s, Ar-C(CH_3)=CH-); 12.52 (1H, broad, s, COOH). EI-MS Mass calculated for $C_{19}H_{15}NO_5$ (m/z): 337.32, found $[M]^+$: 337.10. Elemental analysis found: C, 67.61; H, 4.49; N, 4.10; O, 23.80.

2. Result and Discussion

Oxazine analogues are synthesized by condensing 7-hydroxy-4-methylchromen-2-one with aromatic amines. It was feasible to validate the interpretation of the compound's structures by using spectral analysis methods like ^{1}H NMR and IR. Melting points were taken it was heated by an unrevised scientific process. IR data

supports all functional groups present in title compounds. Formation of Benzopyrone[8,7-e][1,3]oxazines confirmed by removal of the hydroxy group (-OH) at the 7th position & cyclization takes place and supported by the absence of hydroxyl group frequency in IR & peak of -OH group disappears in the ^{1}H NMR of the title compound. Additional studies on a substance's antibacterial and antifungal characteristics are shown in **Table no. 1**, along with activity data. **3d** was discovered to be the most effective against Bacillus subtilis and **3b** in Candida albicans. Escherichia coli was discovered to be sensitive to **3c** and Pseudomonas aeruginosa in **3e**. Compounds **3a** and **3b** were discovered to be effective against Staphylococcus aureus, as shown in **Table no. 1**. Aspergillus fumigatus was more susceptible to the fungicidal effects of the two most potent compounds **3e** and **3f**. The title compounds showed strong inhibitory effects against tested Gram-positive, Gram-negative bacteria and Antifungal compared to standard Gentamycin.



R = a: H
b: m-NO_2,
c: p-NO_2,
d :p-SO_3H,
e: p-CH_3,
f: p-COOH.

Fig. 1: Reaction scheme Synthesis of Benzopyrone[8,7-E][1,3]Oxazines Derivatives.

Antimicrobial Activity:

Zone of Inhibition in mm						
Compound	Bacillus subtilis (gm^{+ve})	Staphylococcus aureus (gm^{+ve})	Escherichia coli (gm^{-ve})	Pseudomonas aeruginosa (gm^{-ve})	Aspergillus fumigatus (fungi)	Candida albicans (fungi)
3a	12mm	32mm	18mm	13mm	16mm	13mm
3b	15mm	33mm	22mm	17mm	12mm	14mm
3c	14mm	16mm	29mm	18mm	18mm	13mm
3d	19mm	10mm	19mm	21mm	20mm	12mm
3e	16mm	15mm	13mm	34mm	22mm	10mm
3f	14mm	16mm	15mm	25mm	23mm	13mm
Gentamicin	10mm	11mm	10mm	11mm	9mm	10mm

Table 1: Zone of inhibition of the synthesized compounds

By using a disc diffusion technique as described by the Kirby-Bauer method [30], the antibacterial and antifungal activity of title compounds was screened against microorganisms. Each synthesized substance was applied to the plates in an aseptic manner after being diluted with DMSO (dimethyl sulfoxide) (0.2 ml of 10 mg/ml). The created compounds were grown on plates using nutrient agar (10mm). To measure the antimicrobial activity, the zones of bacterial and fungal growth inhibition produced by the diffusion of chemicals from the well into the surrounding media were measured using a standard scale after the plates had been incubated for 24 hours at 37°C. The antibacterial and antifungal properties of every produced compound were evaluated against the bacteria Escherichia coli (gm^{-ve}), Pseudomonas aeruginosa (gm^{-ve}), Staphylococcus aureus (gm^{+ve}), Bacillus subtilis (gm^{+ve}) and fungus Aspergillus fumigatus, Candida albicans. The standard control medication in the experiment was gentamicin. Title compounds **3a-f** exhibit excellent efficacy.

ADME Studies:

The physical properties and the ADME parameters (absorption, distribution, metabolism and excretion) using the freely accessible web server Swiss ADME [31]. The results of in silico ADME properties of **(3a-f)** are listed in **Table no. 2.** The molecular weight (MW), the number of hydrogen bond acceptors (nHBA), donors (nHBD), the number of rotatable bonds (nRB) and the topological polar surface area (TPSA) for all the compounds were by the Lipinski's rule of five. The parameters considered to measure the score are lipophilicity (–0.7 < XLOGP3 < 5.0), molecular weight (MW) (150 g mol–1 < MW < 500 g mol–1), polarity (20 Å2 < TPSA < 130 $Å^2$), solubility (0 < log S (ESOL) < 6), saturation (0.25 < Fraction Csp3 < 1) and flexibility (0 < of rotatable bonds < 9).

Table 2: Swiss ADME results of Benzopyrone[8,7-e][1,3]oxazine analogues and standard Ibuprofen and BHT.

COMPOUND	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**Ibuprofen**	**BHT**
MW	293.32	338.31	338.31	373.38	307.34	353.37	206.28	220.35
Heavy atoms	22	25	25	26	23	26	15	16
Aromatic heavy atoms	16	16	16	16	16	16	6	6
Fraction Csp3	0.17	0.17	0.17	0.17	0.21	0.2	0.46	0.6
Rotatable bonds	1	2	2	2	1	2	4	2
H-bond acceptors	3	5	5	6	3	5	2	1
H-bond donors	0	0	0	1	0	1	1	1
MR	88.65	97.47	97.47	98.51	93.62	102.53	62.18	71.97
TPSA	42.68	88.5	88.5	105.43	42.68	79.98	37.3	20.23

iLOGP	2.84	2.49	2.37	1.63	3.1	0	2.17	3.33
XLOGP3	3.28	3.11	3.11	2.03	3.65	3.46	3.5	5.1
MLOGP	3.03	2	2	2.33	3.26	2.85	3.13	4.12
Consensus Log P	3.15	2.57	2.55	2.25	3.49	2.54	3	4.24
Consensus Log S	-4.2	-4.24	-4.24	-3.76	-4.49	-4.53	-3.36	-4.56
ESOL Solubility (mg/ml)	1.86E-02	1.95E-02	1.95E-02	6.53E-02	9.86E-03	1.03E-02	9.09E-02	6.00E-03
GI absorption	High	High	High	High	High	High	High	High
BBB permeant	Yes	No	No	No	Yes	No	Yes	Yes
Pgp substrate	No	No	No	No	No	Yes	No	No
Lipinski violations	0	0	0	0	0	0	0	0
Muegge violations	0	0	0	0	0	0	0	2
PAINS alerts	0	0	1	0	1	0	0	0
Leadlikeness violations	0	0	0	1	1	1	1	2

According to the rule of Five all the synthesized compounds (3A-H) showed a score of 55%, indicating good bioavailability. Some drugs have to be highly water solubility to deliver active ingredients and to estimate this qualitative estimation of solubility log S scale was used: if log S < –10 poorly soluble, < –6 moderately soluble, < –4 soluble, < –2 very soluble and < 0 highly soluble. Based on this predictive model, compounds **(3a-c & 3e-f)** were predicted to be moderately soluble and compounds **(3d)** are predicted to be water-soluble. The lipophilicity and polarity of small compounds are calculated using the brain or intestinal estimated permeation technique(BOILED-Egg), which is offered as an accurate prediction model [32]. The white area denotes passive gastrointestinal absorption, whereas the yellow area denotes passive brain permeability. The derivatives **(3a-f)** have high GI absorption as shown in **Table no. 2**. while compounds **(3a)** and **(3e)** have BBB (blood-brain barrier) permeant as tabulated in **Table no. 2**. When a molecule is digested, there is a chance that dangerous toxicants will enter the bloodstream and the brain because it is blood-brain permeable. The other substances were expected to not permeate the blood-brain barrier.

Figure 2: BOILED-Egg representation of Benzopyrone[8,7-e][1,3]oxazine analogs and standard BHT and Ibuprofen**.**

3. Conclusion

By cyclizing the 7-hydroxy-4-methyl-chromen-2-one with formaldehyde and various aromatic amines, synthesized benzoxazine-2-one derivatives. TLC, IR, ^{1}HMR, and mass spectroscopy are used to examine the synthesized compounds and succeeded. Title compounds inhibit effective antifungal and antibacterial properties. These findings may subsequently be helpful for the ongoing development of fresh antifungal and antibacterial drugs. In silico ADME (Adsorption Distribution Metabolism Excretion) prediction of synthetic compounds has been attempted and title compounds were found to be in the acceptable range and pass the ADME examination, whereas only a few compounds passed the projected toxicity evaluation from boiled egg representation. That assures their absorption in the blood-brain barrier. Our research assures the foundation for the first step in finding prospective new compounds with promising biological activity and minimal toxicity.

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4. References

1. N. Matzanke, W. Lowe, S. Perachon, P. Sokoloff, J. C. Schwartz, H. Stark, Eur. J. Med. Chem., 34, 791, 1999.
2. T. Fukuda, M. Setoguchi, K. Inaba, H. Shoji, T. Tahara, Eur. J. Pharmacol., 196, 299, 1991.

A. S. Bourlot, I. Sanchez, G. Dureng, G. Guillaume, R. Massingham, A. Monteil, E. Winslow, M. D. Pujol, J. Y. Merour, J. Med. Chem., 41, 3142, 1998.

3. E. Blattes, B. Lockhart, P. Lestage, L. Schwendimann, P. Gressens, M. B. Fleury, M. Largeron, J. Med. Chem., 48, 1282, 2005.
4. G. Thuillier, J. Laforest, P. Bessim, J. Bonnet, J. Thuillier, Eur. J. Med. Chem., 10, 37, 1975.
5. J. R. Empfield, K. Russell. Ann, Rep. Med. Chem., 30, 81, 1995.
6. T. Hirata, H. Saito, H. Tomioka, K. Sato, J. Jidoi, K. Hosoe, T. Hidaka, Antimicrob. Agents Chemother., 39, 2295, 1995.
7. H. Matsuoka, N. Ohi, M. Mihara, H. Suzuki, K. Miyamoto, N. Maruyama, K. Tsuji, N. Kato, T. Akimoto, Y.

Takeda, K. Yano, T. Kuroki, J. Med. Chem., 40, 105, 1997.
8. S. Atarashi, H. Tsurumi, T. Fujiwara, I. Hayakawa, J. Heterocycl. Chem., 28, 329, 1991.
9. Thakur. A, Singla. R, Jaitak. V, Eur J Med Chem., 101, 476–495, 2015.
10. Riveiro. ME, De Kimpe. N, Moglioni. A, Vazquez. R, Monczor. F, Shayo. C, Curr Med Chem., 17, 1325–1338, 2010.
11. Meyerhoefer. TJ, Kershaw. S, Caliendo. N, Eltayeb. S, HanawaRomero. E, Bykovskaya. P, Huang V. Marzabadi, CH, De Castro. M, Eur J Org Chem., 2015, 2457–2462, 2015.
12. Mostafavi. H, Najjar. R, Maskani. E, Chem Nat Compd., 49, 423–425, 2013.
13. Nagorichna. IV, Garazd. MM, Garazd. YL, Khilya. VP, Chem Nat Compd., 43, 15–18, 2007.
14. J.N. Joyce, S. Presgraves, I. Renish, S. Borwege, D.H. Osredkar, M. Replogle, M. PazSoldan, M. Mllan, J. Exp. Neurol., 184, 393-407, 2003.
15. F. A. Kerdesky, J. Tetrahedron Lett., 46, 1711-1712, 2005.
16. Taira. J, Ikemoto. T, Yoneyama. T, Hagi. A, Murakami. A, Free Radic Res Commun., 16, 197–204, 1992.
17. Burckhalter. JH, Tendick. FH, et al. J Am Chem Soc., 70, 1363–1373, 1948.
18. Mathew. BP, Kumar. A, Sharma. S, Shukla. PK, Nath. M, Eur J Med Chem., 45, 1502–1507, 2010.
19. Alper-Hayta. S, Aki-Sener. E, Tekiner-Gulbas. B, Eur J Med Chem., 41, 1398–1404, 2006.
20. Macchiarulo. A, Costantino. G, Fringuelli. D, Vecchiarelli. A, Schiaffella. F, Fringuelli. R, Bioorg Med Chem., 10, 3415–3423, 2002.
21. Khan. A, Prasad. S, Parmar. VS, Sharma. SK, J Heterocyclic Chem., 53, 1264–1275, 2016.
22. Verma. V, Singh. K, Kumar. D., et al. Eur J Med Chem., 56, 195–202, 2012.
23. P. Zhang, E. A. Terefenko, A. Fensome, Z. Zhang, Y. Zhu, J. Cohen, R. Winneker, J. Wrobel, J. Yardley, Bioorg. Med. Chem. Lett., 12, 787, 2002.
24. Fringuelli. R, Schiaffella. F, Bistoni. F, Pitzurra. L, Vecchiarelli, Bioorg. Med. Chem., 6, 103, 1998.
25. Pitzurra. L, Fringuelli. R, Perito. S, Schiaffella. F, Barluzzi. R, Bistoni. F, Vecchiarelli. A, Antimicrob. Agents Chemother., 43, 2170, 1999.
26. Schiaffella. F, Guarraci. A, Fringuelli. R, Pitzurra. L, Bistoni. F, Vecchiarelli. A, Med. Chem. Res., 9, 291, 1999.
27. Fringuelli. R, Pietrella. D, Schiaffella. F, Guarraci. A, Perito. S, Bistoni. F, Vecchiarelli. A, Bioorg. Med. Chem., 10, 1681, 2002.
28. Loncaric. M, Gaso-Sokac. D, Jokic. S, Molnar. M, Biomolecules., 10, 151, 2020.
29. Bauer. A. W, Kirby. W. M, M. Sherris. J, C. Turck. M, Am J Clin Path., 45(4), 493-6, 1966.
30. Daina. A, Zoete. V, Nature: Scientific Reports., 7(1), 42717, 2017.
31. Diana. A, Zoete. V, Chem Med Chem., 11, 1117-1121, 2016.