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Research Article

SYNTHESIS AND ANTIBACTERIAL EVALUATION OF 3-ARYL INDAZOLES

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ABSTRACT

A series of 3-aryl indazoles **4a-4j** were obtained by Pd catalyzed Suzuki coupling reaction. All the substrates were obtained in good yields under moderate reaction conditions. Next, the prepared compounds **4a-4j** were screened for their antibacterial activity. The anti-bacterial activities of the prepared compounds were investigated against three bacterial strains i.e. *Xanthomonas campestris*, *Escherichia coli*, *Bacillus megaterium*. The anti-bacterial evaluation studies of these 3-aryl indazoles revealed that some of these test compounds possess moderate *in vitro* antibacterial activity.

Keywords: Indazoles, Palladium, Antibacterial activity.

1. INTRODUCTION

Nitrogen-containing heterocycles are the main scaffolds of many biologically significant compounds and medicinal products due to their ubiquitous nature. These nitrogen-containing heterocycles are used in some of the world's most popular pharmaceuticals [1]. Indazole and its derivatives are now recognised as a significant family of nitrogen-containing heterocycles with a wide range of biological, agricultural and industrial applications [2]. Because of their broad range of biological properties, such as antimicrobial [3], anti-inflammatory [4], anti-HIV [5], anti-angiogenesis [6], antihypertensive [7], anticancer [8], neuroprotective [9], anti-protozoal [10], and anti-tubercular [11] activities, indazole analogues have gotten a lot of attention in the past and in recent years. Several Estrogen [12] and 5-HT1A receptors also been identified have the indazole nuleus [13].

In addition, in current drug design and discovery, indazoles are useful bioisosters for benzimidazoles and indoles [14]. Number of indazole derivatives, in particular, have already been reported to be excellent antibacterial and antimicrobial agents (Fig. 1) [15-20].

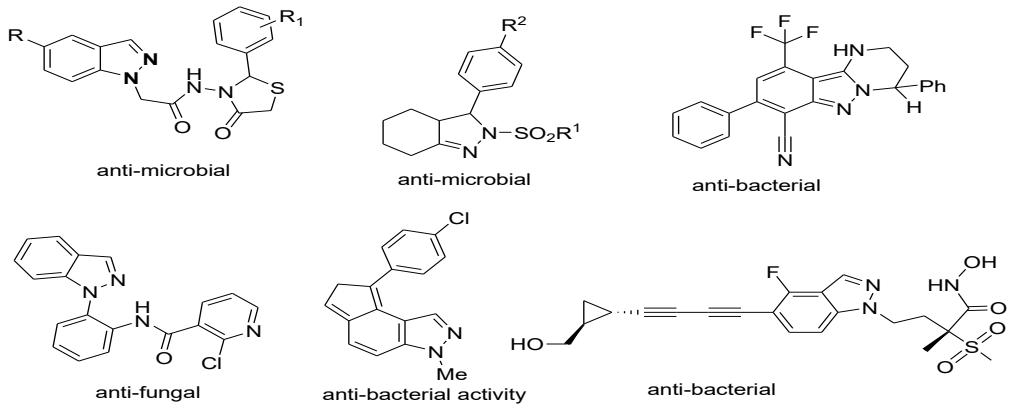


Fig. 1: Some indazole derivatives with antimicrobial activity

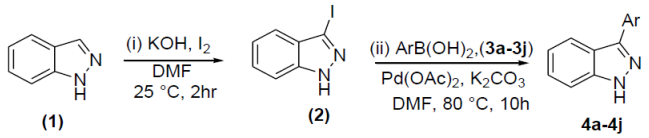
Furthermore, the use of many antimicrobial drugs is limited not only by rapidly developing drug resistance, but also by the current state of therapy for fungal and bacterial infections, which is inadequate. As a result, one of the most important areas of antimicrobial research is the discovery of novel chemicals that are

resistant to bacteria and fungi. The increasing resistance to conventional antimicrobial drugs has necessitated the research and development of novel themes for infection therapy that have several mechanisms of action that can target both resistant and sensitive microbial strains [21]. One of the promising strategies for overcoming the resistance problem is to screen potential antibacterial agents among new classes of chemical compounds. Taking all of the aforementioned into account, chemists from all over the world have documented several ways for constructing indazole heterocycles. Very recently Hari Babu *et al* reported [22] the synthesis and anticancer activity of 3-aryl indazoles and *N*-methyl-3-aryl-indazole derivatives using Pd catalyst. In this present work we followed the same method for the synthesis of 3 -aryl-indazoles **4a-4j** and evaluated their antibacterial properties**.**

2. EXPERIMENTAL

2.1. Synthesis of *N*-methyl-3-aryl indazoles 4a-4j

Hari Babu *et al* [22] recently established the Pd-catalyzed synthesis of N-methyl-3aryl-indazole derivatives from indazole *via* iodination and Pd promoted Suzuki-mayura coupling reaction.



Scheme 1: Synthetic route for 3-aryl indazoles

The same procedure was used to prepare the titled 3-aryl indazoles **4a-4j.** The synthetic results of compounds 4a-4j were presented in table 1.

Table 1: Synthetic results of compounds 4a-4j

Com-pound	Ar	Yield (%)	M.P (°C)
4a	Phenyl	65	114-116 °C
4b	Napthyl	67	136-138 °C
4c	4-floro phenyl	55	112-113 °C
4d	Pyridin-4-yl	63	101-103 °C
4e	Pyridin-3-yl	62	184-186 °C
4f	4-methoxyphenyl	70	85-87 °C
4g	4-(methylthio)phenyl	70	123-125 °C
4h	2-methoxyphenyl	68	115-116 °C
4i	4-hydroxy phenyl	60	121-123 °C
4j	*N,N*-dimethylbenzamide	60	116-117 °C

The functionalization at the C-3 position of indazoles is of immense interest. Thus inspired by the literature of Suzuki couplings, in the present work, we append the aromatic moieties after iodination at C-3 position of indazole, followed by Pd-catalyzed C-C bond formation to obtain 3-aryl-1*H*-indazoles (**3a–3j**). The 3-iodo indazole (**2**) is the key intermediate in this process, which is obtained by the iodination of indazole (1) using KOH/I_2 in DMF. Next, Most of the synthesized compounds originated with the Pd-catalyzed aryl coupling reaction of the 3-iodoindazole (**2**) with diverse aromatic boronic acids in dimethylformamide (DMF) which yields 3-aryl-1*H*-indazoles (**3a-3j**) in 55-70% of yields, and the results are indicated in Fig. 2.

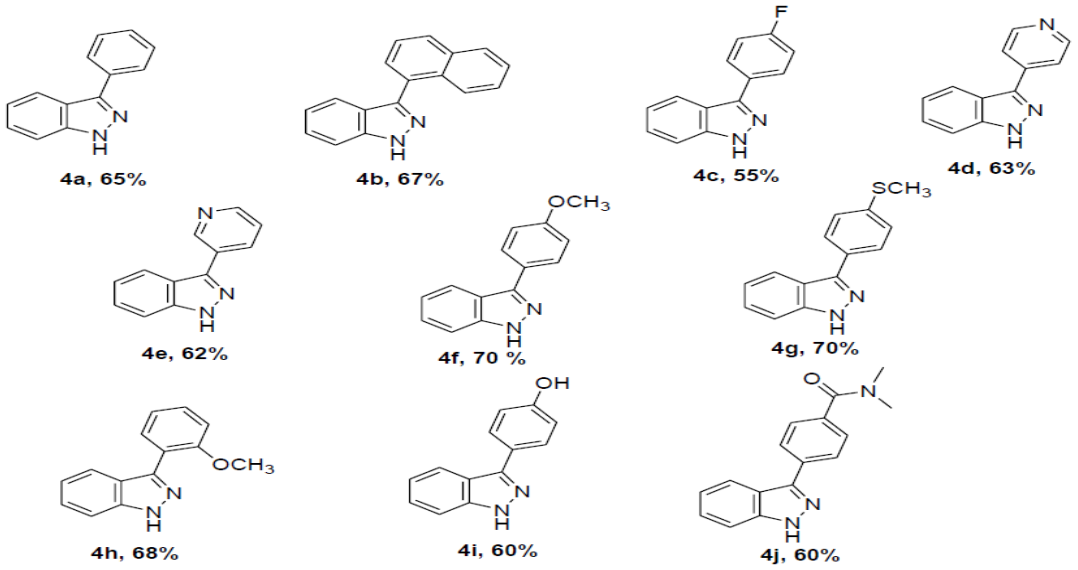


Fig. 2: Prepared *N*-methyl-3-aryl indazoles 4a-4j

2.2. Antimicrobial activity

The antibacterial activity of the synthesized **4a-j** compounds was next tested against three medicinally important bacterial strains: *Xanthomonas campestris (MTCC-2286), Escherichia coli (MTCC-1687)* and *Bacillus megaterium (MTCC-428).* The *in vitro* antimicrobial activity of produced compounds **4a-j** was estimated using the well diffusion method [23]. Antibacterial testing was performed on one-day-old bacterial cultures. The pour plate method was used to make bacterial culture plates, which involved pouring roughly 0.3 mL of each bacterial solution into sterile petri plates before the addition of molten state nutrient agar. With a sterile cork borer, 8 mm diameter wells were drilled after solidification. 2 mg of each compound was dissolved in 500 mL DMSO to make the test samples. 100 litres of the sample were poured into the wells. For each 24 hours period, the plates were incubated at 37°C. After incubation, the diameter of the inhibitory zone was measured. For each sample and bacterial species, three duplicates were kept. As a positive control, a standard antibiotic, Streptomycin, was used at a similar concentration. The average inhibition zone was determined and compared to the standard zone. Antimicrobial activity against different organisms was examined using a similar approach.

3. RESULTS AND DISCUSSIONS

3.1. Spectral characterization of the synthesized compounds 4a-4j

3.1.1. 3-Phenyl-1H-indazole (4a)

Yield:65%; m.p. 114-116 °C; IR (KBr, υmax, cm^{-1}): 3691 (OH str), 3440 (b,NH str), 2991 (Ar=CH str), 2897 (CH str), 1601, 1545, 1498 (Ar C=C str), 1450 (C=N str), 1233 (N-N str); NMR:1H (500 MHz, CDC_{l3}):δ = 11.18 (b, 1H), 8.08-8.04 (m, 3H), 7.58 (t, J = 7.5, 2H), 7.49-7.46 (m, 1H), 7.43-7.37 (m, 2H), 7.29-7.25 (m, 1H); 13C-NMR:(125 MHz, DMSO): δ = 145.8, 141.7, 133.6, 128.9, 128.2, 127.7, 126.8, 121.4, 121.1, 121.0, 110.2; m/z (ESI-MS) 195.23 $(M + H)^+$.

3.1.2. 3-(Naphthalen-1-yl)-1H-indazole (4b)

Yield:65%; m.p. 136-138 °C; IR (KBr, υmax, $cm^{-}1$): 3446 (b,NH str), 2929 (Ar=CH str), 1595, 1581, 1506 (ArC=C str), 1418 (C=N str), 1246 (N-N str); NMR:^{1}H (500 MHz, $CDCl_3$):δ = 12.25 (b, 1H), 8.32-8.31 (m, 1H), 8.01-7.98 (m, 2H), 7.79-7.67 (m, 2H), 7.57-7.52 (m, 2H), 7.48-7.39 (m, 2H), 7.21 (t, J = 6.7, 1H), 6.93-6.92 (m, 1H). ^{13}C-NMR (125 MHz, CDCl3): δ = 143.4, 134.1, 131.9, 131.3, 128.8, 128.2, 128.1, 126.7, 126.4, 126.3, 126.1, 125.4, 121.9, 121.3, 110.9:m/z (ESI-MS) 245.05 $(M + H)^+$.

3.1.3. 3-(4-Fluorophenyl)-1H-indazole(4c)

Yield:65%; **m**.p. 112-113 °C; IR (KBr, υmax, cm^{-1}): 3406 (NH str), 3078 (ArH str), 2924 (ArH str), 1625, 1563 (ArC=C str), 1440 (C=N str), 1370 (C=N str), 814 (C-F str); 1H NMR (300 MHz, $CDCl_3$):δ 8.05 (m, 3H, ArH), 7.45 (m, 2H, ArH), 7.25 (m, 1H, ArH); 7.21 (m, 2H, ArH); 13C NMR (75 MHz, $CDCl_3$):δ = 151.0, 147.2, 138.5, 130.1, 128.8, 126.2, 124.4, 122.1, 121.7, 113.8; m/z (ESI-MS) 213.27 $(M + H)^+$.

3.1.4. 3-(Pyridin-4-yl)-1H-indazole (4d)

Yield:65%; m.p. 101-103 °C; IR (KBr, υmax, cm^{-1}): 3430 (NH str), 3032 (ArCH str), 1584, 1549, 1491(ArC=C str), 1373 (C=N str), 1211 (N-N str); 1H NMR (300 MHz, $CDCl_3$):δ 7.73-7.62 (m, 4H, Ar-H), 7.62 (d, J = 8 Hz, 2H, Ar-H); 7.45 (d, J = 9 Hz, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$):δ = 148.8, 143.6, 138.5, 130.1, 128.8, 126.2, 124.4, 122.1, 121.7, 112.3; m/z (ESI-MS) 196.26 $(M + H)^+$.

3.1.5. 3-(Pyridin-3-yl)-1H-indazole (4e)

Yield:65%; m.p. 184-186 °C; IR (KBr, υmax, cm^{-1}): 3409 (b, NH str), 3066 (ArCH str), 1589, 1560, 1512 (ArC=C str), 1340 (C=N str), 1216 (N-N str); ^{1}H NMR (300 MHz, $CDCl_3$):δ 10.92 (s, 1H, NH), 9.25 (s, 1H, ArH), 8.65 (d, 1H, J = 7.5, Ar-H), 8.42 (d, 1H, J = 8.4 Hz, ArH); 8.05 (d, J = 7.5 Hz, 1H, Ar-H) 7.22-7.64 (m, 4H, ArH); ^{13}C NMR (125 MHz, $CDCl_3$):δ = 148.5, 139.6, 139.2, 128.8, 127.5, 126.2, 125.2, 124.4, 124.3, 123.0, 122.6, 122.2; m/z (ESI-MS) 196.26 $(M + H)^+$.

3.1.6. 3-(4-Methoxyphenyl)-1H-indazole(4f)

Yield:65%; m.p. *85-87 °C; IR (KBr, υmax, cm^{-1}): 3429 (NH str), 3054 (ArH str), 2927 (CH str), 1583, 1487 (ArC=C str), 1438 (*C=N str), 1375 (C=N str), 1169 (C-O-C str); ^{1}H NMR (300 MHz, $CDCl_3$):δ 8.26 (s, 1H, NH), 8.03 (d, 1H, J = 8.0, ArH), 7.85 (dd, 2H, J = 10.5, ArH), 7.46 (m, 2H, ArH); 7.22 (m, 1H, ArH), 7.03 (dd, 2H, J = 10.4, ArH); 13C NMR (75 MHz, $CDCl_3$):δ = 159.6, 145.7, 141.8, 128.9, 126.6, 126.2, 121.1, 121.1, 114.4, 110.3, 55.3; m/z (ESI-MS) 225.31 $(M + H)^+$.

3.1.7. 3-(4-(Methylthio)phenyl)-1H-indazole (4g)

Yield:65%; m.p. 123-125 °C; IR (KBr, υmax, cm^{-1}): 3378 (NH str), 3052 (ArH str), 2925 (CH str), 1600, 1521 (ArC=C str), 1346 (C=N str), 1106 (C-S-C str); 1H NMR (300 MHz, CDCl3):δ 8.16 (s, 1H, NH), 7.92 (d, 2H, J = 7.5, ArH), 7.52 (d, 1H, J = 8.4, ArH), 7.38-7.7.51 (m, 4H, ArH); 7.23 (s, 1H, ArH), 2.45 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 160.0, 145.7, 141.8, 134.7, 129.9, 126.8, 121.4, 121.0, 120.2, 114.2, 112.9, 110.3, 55.3.; m/z (ESI-MS) 241.30 $(M + H)^+$.

3.1.8. 3-(2-Methoxyphenyl)-1H-indazole (4h)

Yield:65%; m.p. 115–116 °C; IR (KBr, υmax, cm^{-1}): 3325 (b, NH str), 3025 (ArCH str), 2921 (CH str), 1659, 1513, 1437(ArC=C str), 1370 (C=N str), 1212 (N-N str), 1148 (C-O-C str); ^1H NMR (300 MHz, CDCl3):δ 7.81 (s, 1H, NH), 7.72 (d, 1H, J = 7.5 Hz, ArH), 7.52 (d, J = 8.5 Hz, 1H, Ar-H) 7.42 (m, 2H, ArH); 7.21 (m, 1H, ArH), 7.09 (m, 1H, ArH), 7.02 (d, 1H, J = 10.2 Hz, ArH), 3.8 (s, 3H, OCH_3).^{13}C NMR (125 MHz, $CDCl_3$):δ = 157.3, 143.0, 141.4, 131.4, 129.7, 126.3, 122.2, 122.0, 120.9, 120.5, 111.4, 110.4, 55.4; m/z (ESI-MS) 225.30 $(M + H)^+$.

3.1.9. 4-(1H-indazol-3-yl)phenol(4i)

Yield:65%; m.p. 121-123 °C; IR (KBr, υmax, $cm^{-}1$): 3414 (OH str), 3315 (b,NH str), 2925 (Ar=CH str), 1651, 1560, 1505(ArC=C str), 1414 (C=N str), 1219 (N-N str), 1093 (C-O str); 1H NMR (300 MHz, $CDCl_3$):δ 13.12 (s, 1H, PhOH), 9.62 (s, 1H, NH), 8.01 (d, 1H, J = 8.4 Hz, ArH), 7.82 (d, J = 7.5 Hz, 2H, Ar-H), 7.49 (d, J = 10.2, 1H, ArH), 7.39 (d, J = 10.4, 1H, ArH), 7.22 (d, J = 8.0 Hz, 1H, ArH), 6.96 (m, 2H, ArH); 13C NMR (75 MHz, $CDCl_3$):δ = 158.4, 151.4, 148.6, 138.8, 132.5, 134.8, 128.7, 125.9, 122.1, 113.5, 104.1; m/z (ESI-MS) 211.30 $(M + H)^+$.

3.1.10. 4-(1H-indazol-3-yl)-N,N-dimethylbenzamide (4j)

Yield:65%; m.p. 116-117 °C; IR (KBr, υmax, cm^{-1}): 3456 (b, NH str), 2933 (Ar=CH str), 2835 (CH str), 1817 (CO str), 1649, 1530, 1488(ArC=C str), 1386(C=N str), 1210 (N-N str); ^1H NMR (500 MHz, $CDCl_3$):δ 9.86 (s, 1H, NH), 8.09 (m, 3H, ArH), 7.61 (m, 3H, Ar-H), 7.45 (d, J = 10.5 Hz, 1H, ArH); 7.28 (s, 1H, ArH), 3.12 (s, 6H, CH_3); ^{13}C NMR (100 MHz, CDCl3):δ = 166.3, 145.7, 135.2, 132.2, 130.4, 128.7, 128.6, 128.1, 127.9, 127.3, 126.5, 114.1, 36.4; m/z (ESI-MS) 266.35 $(M + H)^+$.

3.2. Antibacterial activity

By using the well diffusion method, the *in vitro* antimicrobial activity of synthesized N-Methyl-3-aryl substituted indazoles **4a-4j** was tested. *Xanthomonas campestris, Bacillius megaterium* (gramme positive), and *Escherichia coli* (gram negative) starins were used to evaluate the anti bacterial activity of the prepared compounds. As per the results, Table 2 shows the diameter of the zone of inhibition exhibited by the tested motifs for the bacterial strains at a concentration of 100 µL. The bilogical study reveal that the studied compounds **4a-4j** have good antibacterial properties. As per the results, the exhibited diameter of zone of inhibition by the test compounds againt the bacterial strains at a concentration of 100 µL is shown in Table 2.

Table 2: Antimicrobial evaluation of novel compounds (5a-5j)

Compound	*Zone of inhibition in mm*		
	XC[a]	***EC***[b]	***BM***[c]
4a	18	11	12
4b	11	09	--
4c	10	11	08
4d	12	10	--
4e	12	08	08
4f	17	10	08
4g	10	10	08
4h	11	10	--
4i	18	10	11
4j	10	12	12
Streptomycin (Standard as positive control)	**28**	**39**	**37**

XC[a] Xanthomonas campestris; EC[b] Escherichia coli; BM[c] Bacillus megaterium; - - :no zone of inhibition.

This screening outcome reveals that, compounds **4a**, **4f**, and **4i** demonstrated higher efficacy against *Xanthomonas campestris*, with zones of inhibition of 18, 18, and 17 mm, respectively, as compared to the zone of inhibition of standard streptomycin, which was 28 mm. With zone of inhibitions of 12, 12, and 11 mm, compounds **4a**, **4c**, and **4j** exhibited remarkable efficacy against the pathogen *Bacillus megaterium*. The compounds **4a**, **4j**, and **4i** are moderately active against *Escherichia coli* with zones of inhibition of 11 mm, 12 mm, and 11mm

respectively, when compared to the standard of streptomysin which has a zone of inhibition of 38 mm. According to the results of the zone of inhibition investigation, it was concluded that (i) the superior anti-bacterial activity of the compounds **4f**, **4i** are due to the presence of electron donating methoxy and hydroxyl groups at 4th position of the phenyl ring; (ii) indazole with unsubstituted phenyl at C_3-position (**4a)** showed good anti-bacterial activity also.

3.3. Minimum Inhibitory Concentration (MIC)

The authors used the above results to test the compounds **4a-4j** for the minimum inhibitory concentration (MIC) of those compounds with a high zone of inhibition in the above test, in order to control the microorganisms *Xanthomonas campestris*, *Escherichia coli*, and *Bacillius megaterium*. The MIC (minimal inhibitory concentration) was determined by measuring the lowest concentration of the drug required to stop the bacterium from growing. Table 3 summarises the MIC values for the substances examined.

The MIC of compound **4j, 4j,** and **4i** to prevent *Bacillius megaterium* growth was 100 µL, according to the aforementioned antimicrobial screening data. *Escherechia coli* growth was also inhibited at a MIC of 100 µL for compounds **4j**, **4a**, and **4c**. However, a MIC of 25 µL of **4a**, **4f**, and **4i** compounds was sufficient to suppress *Xanthomonas campestris.*

From the above investigation it was revealed that a) motifs **4a, 4f, 4i** exhibited superior antibacterial activity at low MIC levels to control the gram negative bacterium, *Xanthomonas campestris.* (b) scaffolds **4a, 4j, 4i** and **4c** exhibited potential anti-bacterial activity at low MIC levels to control the pathogenic strains *Bacillus Megaterium and Escherechia coli.*

Table 3: Minimum Inhibitory Concentration test for compounds 4a-4j *Bacillus megaterium:*

Compound	Concentration of the compound (µL)			
	Growth (OD) of the organism at different concentrations of compound			
	25µL	**50µL**	**75µL**	**100µL**
4j	0.183	0.123	0.072	--
4a	0.205	0.134	0.083	--
4i	0.221	0.146	0.097	--
Escherechia coli				
4j	0.192	0.145	0.081	--
4a	0.197	0.151	0.088	--
4c	0.198	0.157	0.090	--
Xanthomonas campestris:				
4a	0.110	--	---	--
4f	0.376	--	--	--
4i	0.496	--	---	--

- - : no zone of inhibition

4. CONCLUSIONS

The development of heterocycles with medicinal value using simple reagents is inspired by the synthesis of indazole derivatives. Initially, various 3-aryl indazole analogues were proposed and produced. Following that, the antibacterial activity of the analogues was investigated. The majority of the compounds are effective against the teated three bacterial strains. Against several bacterial strains, the compounds **4a**, **4i**, and **4j** showed excellent inhibitory action. Finally, we believe that this group of indazole derivatives presents an interesting profile for further research, particularly in the field of antimicrobial research.

5. REFERENCES

1. Mills AD, Nazer MZ, Haddadin MJ, Kurth MJ. *J. Org. Chem.,* 2006; **71(7):**2687-2689.
2. Zhang T, Bao W. *J. Org. Chem.,* 2013; **78(3):**1317-1322.
3. Li X, Chu S, Feher VA, Khalili M, Nie Z, Margosiak S, et al. *J. Med. Chem.,* 2003; **46(26):**5663-5673.
4. Villanueva JP, Mulia LY, Sanchez IG. *Molecules,* 2017; **22(11):**1864
5. Kim SH, Markovitz B, Trovato R, Murphy BR, Austin H, Willardsen AJ, et al. *Bioorg. Med. Chem. Lett.*, 2013; **23(10):** 2888-2892.

6. Sun Y, Shan Y, Li C, Si R, Pan X, Wang B, Zhang J. *Eur. J. Med. Chem.,* 2017; **141:**373-385.
7. Saczewski F, Kornicka A, Rybczyn′ska A, Hudson AL, Miao SS, Gdaniec M, et al. *J. Med. Chem.,* 2008; **51(12):**3599-3608.
8. Chu YY, Cheng HJ, Tian, ZH, Zhao JC, Li G, Chu YY, et al. *Chem. Biol. Drug Des.*, 2017; **90(4):**609-617.
9. Lin YC, Chou LC, Chen SC, Kuo SC, Huang LJ. *Bioorg. Med. Chem. Lett.,* 2009; **19(12):**3225-3228.
10. Gerpe A, Aguirre G, Boiani L, Cerecetto H, González M, Azar CO, et al. *Bioorg. Med. Chem.,* 2006; **14(10):**3467-3480.
11. Karalı N, Gürsoy A, Kandemirli F, Shvets N, Kaynak FB, Ozbey S, et al. *Biorg. Med. Chem.,* 2007; **15(17):**5888-5904.
12. Angelis MD, Stossi F, Carlson KA, Katzenellenbogen BS, Katzenellenbogen JA. *J. Med. Chem.,* 2005; **48(4):**1132-1144.
13. Andreonati S, Sava V, Makan S, Kolodeev G. *Pharmazie,* 1999*;* **54(2):**99-101.
14. Clutterbuck LA, Posada CG, Visintin C, Riddal DR, Lancaster B, Gane PJ, et al. *J. Med. Chem.*, 2009; **52(9):**2694-2707.
15. Ghaemi M, Pordel M. *Chem. Heterocycl. Comp.,* 2016; **52:** 52-57.
16. Minu M, Thangadurai A, Wakode SR, Agrawal SS, Narasimhan B. *Bioorg Med Chem Lett.,* 2009; **19(11):** 2960-2964.
17. Yakaiah T, Lingaiah BPV, Naraiah B, Kumar KP, Murthy US. *Eur J Med Chem.*, 2008; **43(2):**341-347.
18. Actelion Pharmaceuticals Ltd., US 9, 2017; **624:**206.
19. Du SJ, Lu HZ, Yang DY, Li H, Gu XL, Wan C, et al. *Molecules,* 2015; **20(3):**4071-4087.
20. Holmes A, Moore L, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. *Lancet,* 2016; **387(10014):**176-187.
21. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. *Nat. Rev.* Drug *Discov.,* 2007; **6:**29-40.
22. Rao SJM, Murthy BSN, Raghuram M, Adil SF, Rafi SM, Alduhaish O, et al. *Applied Sciences,* 2020; **10(11):**3792.
23. Valgas C, DSouza SM Smania, EF, Smania AJ. *Brazilian Journal of Microbiology,* 2007; **38:**369-380.