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ARTICLE

Reevaluating the synthesis of 2,5-disubstituted-1*H*-benzimidazole derivatives by different green activation techniques and their biological activity as antifungal and antimicrobial inhibitor

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Abstract

A comparative study concerned with the preparation of diversely substituted-1*H*-benzimidazole under different green activation techniques and conventional methods is reported. Data are collected for infrared, ultrasound, microwave, and simultaneous irradiation with US and IR sources, as this last strategy shows an important improvement. Further, the small library of potentially bioactive benzimidazole **17-76** synthesized was screened as an antifungal and antimicrobial agent. Strong activity against *Candida albicans* and *Staphylococcus aureus* was observed. Remarkably, 2-(4-aminophenyl)-5-phenylamino-1*H*-benzimidazole **63** resulted better than that of reference drugs miconazole with a zone of inhibition up to 42 mm. Likewise, 2-(2-aminophenyl)-1*H*-benzimidazole **21** showed substantial antimicrobial activity against MRSA strain. When assayed by the microdilution method, this azaheterocyclic compound presented a minimum inhibitory concentration (MIC) ≥ 16.4 μg/100 mL and a bacterial percentage reduction of 96%.

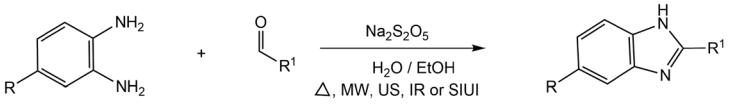
1 | INTRODUCTION

Benzimidazole motif belongs to a unique class of privileged scaffold that forms an integral part of many drugs and candidate drugs that exhibit a wide range of pharmacological profile, such as anticancer,[1] antimicrobial,[2] antiviral,[3] and antifungal.[4] In this regard, the benzo-fused 1,3-diazole skeleton has been considered as pivotal building blocks for the discovering of novel pharmaceutically important lead molecules

with a skeleton of pyrrolo[1,2-*a*]benzimidazole and aziridinylpyrrolo[1,2-*a*]benzimidazolequinone biologically active as DNA cleaving agent and alkylating agent of DNA phosphate backbone.[5] Therefore, implementation and assessment of hi-tech synthetic protocols able to afford concise preparation of structurally simple and complex scaffolds, featuring mild reaction conditions, high efficacy, short time, selectivity, broad substrate scope, and low environmental charge continue attracting the attention of medicinal and organic chemists.

Even though the reductive cyclization of *N*-(4-methyl-2-nitrophenyl)acetamide with metallic reducing agents[6] has been known as one of the first conventional approaches disclosed for the preparation of benzimidazole scaffold, the condensation of *o*-phenylenediamines with carboxylic acids along with esters and lactones[7] stands as the most significant strategy implemented in organic chemistry. Nonetheless, this strategy suffers from several drawbacks wherein highlight handling of highly toxic solvent,[8,9] harsh acid condition, high temperature, and prolonged time of reaction that restrict the employment of acid-sensitive inputs. By contrast, the *o*-phenylenediamine approach catalyzed by I_2/KI, $FeCl_3/O_2$, $FeCl_2$/$FeCl_3$,[10,11] and nitroreductive cyclization of *o*-nitroanilines derivatives with $Na_2S_2O_4$[12] has recently emerged as important variants for the synthesis of 1*H*-benzimidazoles where arenecarbaldehydes substrates have nicely replaced the acid component. Notwithstanding their well-known synthetic applications, this one-pot strategy has remained scarcely explored by eco-friendly reaction techniques as well as combined green techniques despite their ever-increasing interest as a frontline tool highly valued in organic synthesis.

In this context, we have reported the less know combined use of the US with IR (simultaneous irradiation with US and IR [SIUI]) as an improved tool to promote S_NAr reactions.[13] Our aim is to show how these two important green techniques may be combined to provide a simple, cost-effective, and reliable method for application in organic chemistry. Thus, herein, we present the results obtained in the synthesis of a small library of diversely decorated 1*H*-benzimidazoles **17-76** from a mixture of *o*-phenylenediamine **1-6** and arenecarbaldehyde **7-16** in presence of sodium metabisulfite ($Na_2S_2O_5$) dissolved in a mixture of protic polar solvents (H_2O/EtOH) using SIUI sources, besides their comparison with three of the most important nonconventional methodologies (ultrasound [US], infrared [IR], and microwave [MW])



1 R = H
2 R = NO_2
3 R = NH_2
4 R = N=NPh
5 R = NHPh
6 R = $NHCH_2CH_2OH$

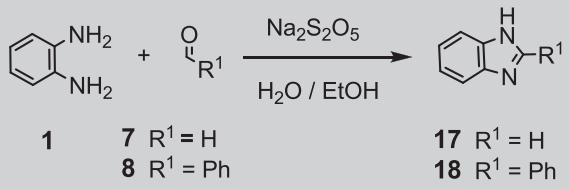
7 R^1 = H
8 R^1 = Ph
9 R^1 = 3,4-$(OMe)_2$Ph
10 R^1 = 4-$N(Me)_2$Ph
11 R^1 = 2-NH_2Ph
12 R^1 = 3-NH_2Ph
13 R^1 = 4-NH_2Ph
14 R^1 = 2-NO_2Ph
15 R^1 = 3-NO_2Ph
16 R^1 = 4-NO_2Ph

17	R = H	R^1 = H	**47**	R = N=NPh	R^1 = H
18	R = H	R^1 = Ph	**48**	R = N=NPh	R^1 = Ph
19	R = H	R^1 = 4-$N(Me)_2$Ph	**49**	R = N=NPh	R^1 = 4-$N(Me)_2$Ph
20	R = H	R^1 = 3,4-$(OMe)_2$Ph	**50**	R = N=NPh	R^1 = 3,4-$(OMe)_2$Ph
21	R = H	R^1 = 2-NH_2Ph	**51**	R = N=NPh	R^1 = 2-NH_2Ph
22	R = H	R^1 = 3-NH_2Ph	**52**	R = N=NPh	R^1 = 3-NH_2Ph
23	R = H	R^1 = 4-NH_2Ph	**53**	R = N=NPh	R^1 = 4-NH_2Ph
24	R = H	R^1 = 2-NO_2Ph	**54**	R = N=NPh	R^1 = 2-NO_2Ph
25	R = H	R^1= 3-NO_2Ph	**55**	R = N=NPh	R^1 = 3-NO_2Ph
26	R = H	R^1 = 4-NO_2Ph	**56**	R = N=NPh	R^1 = 4-NO_2Ph
27	R = NO_2	R^1 = H	**57**	R = NHPh	R^1 = H
28	R = NO_2	R^1 = Ph	**58**	R = NHPh	R^1 = Ph
29	R = NO_2	R^1 = 4-$N(Me)_2$Ph	**59**	R = NHPh	R^1 = 4-$N(Me)_2$Ph
30	R = NO_2	R^1 = 3,4-$(OMe)_2$Ph	**60**	R = NHPh	R^1 = 3,4-$(OMe)_2$Ph
31	R = NO_2	R^1 = 2-NH_2Ph	**61**	R = NHPh	R^1 = 2-NH_2Ph
32	R = NO_2	R^1 = 3-NH_2Ph	**62**	R = NHPh	R^1 = 3-NH_2Ph
33	R = NO_2	R^1 = 4-NH_2Ph	**63**	R = NHPh	R^1 = 4-NH_2Ph
34	R = NO_2	R^1 = 2-NO_2Ph	**64**	R = NHPh	R^1 = 2-NO_2Ph
35	R = NO_2	R^1 = 3-NO_2Ph	**65**	R = NHPh	R^1 = 3-NO_2Ph
36	R = NO_2	R^1 = 4-NO_2Ph	**66**	R = NHPh	R^1 = 4-NO_2Ph
37	R = NH_2	R^1 = H	**67**	R = $NHCH_2CH_2OH$	R^1 = H
38	R = NH_2	R^1 = Ph	**68**	R = $NHCH_2CH_2OH$	R^1 = Ph
39	R = NH_2	R^1 = 4-$N(Me)_2$Ph	**69**	R = $NHCH_2CH_2OH$	R^1 = 4-$N(Me)_2$Ph
40	R = NH_2	R^1 = 3,4-$(OMe)_2$Ph	**70**	R = $NHCH_2CH_2OH$	R^1 = 3,4-$(OMe)_2$Ph
41	R = NH_2	R^1 = 2-NH_2Ph	**71**	R = $NHCH_2CH_2OH$	R^1 = 2-NH_2Ph
42	R = NH_2	R^1 = 3-NH_2Ph	**72**	R = $NHCH_2CH_2OH$	R^1 = 3-NH_2Ph
43	R = NH_2	R^1 = 4-NH_2Ph	**73**	R = $NHCH_2CH_2OH$	R^1 = 4-NH_2Ph
44	R = NH_2	R^1 = 2-NO_2Ph	**74**	R = $NHCH_2CH_2OH$	R^1 = 2-NO_2Ph
45	R = NH_2	R^1 = 3-NO_2Ph	**75**	R = $NHCH_2CH_2OH$	R^1 = 3-NO_2Ph
46	R = NH_2	R^1 = 4-NO_2Ph	**76**	R = $NHCH_2CH_2OH$	R^1 = 4-NO_2Ph

SCHEME 1 2-mono- and 2,5-disubstituted-1*H*-benzimidazole **17-76** synthesized by conventional and unconventional conditions in presence of sodium metabisulfite

and thermal method (Δ). In addition to screening their antifungal and antibacterial activity against *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 8739), and *Staphylococcus aureus* (ATCC 6538 and ATCC43300) strain. Interestingly, benzimidazoles **58**, **63**, and **66** showed meaningful inhibitory activities against *C albicans*, whereas compounds **22** and **21** exhibit notable activities against MRSA microorganism.

TABLE 1 Reaction times and yields of benzimidazole **17** and **18** estimated under conventional and nonconventional method



1 **7** R^1 = H; **8** R^1 = Ph → **17** R^1 = H; **18** R^1 = Ph

Entry	Product	Δ[e]	IR[e]	US[a]	SIUI[b]	MW[c]	Mp (°C) Exp/lit.
			Time (h)/yield (%)				
1	**17**	10/90	0.25/70	0.41/80	0.15/79	0.05/87	232-234/235-236[14]
2	**18**	4.0/96	0.66/85	0.15/93	0.03/90	0.016/88	287-299/288-290[15]
2′	**18**	—	0.66/78(80)	0.15/64 (42)[d]	0.03/86 (51)	—	

Note. Reaction condition: *o*-phenylenediamine (5 mmol), benzaldehyde or formaldehyde (5.5 mmol), $Na_2S_2O_5$ (5 mmol within 5-8 mL), and ethanol 5 mL were subjected to react at 74 ± 2°C.

Abbreviations: IR, infared; MW, microwave; SIUI, simultaneous irradiation with US and IR; US, ultrasound.

[a]Reactions were obtained by exposing the reactants to 2-minute cycling pulse sequences of US (with 5-min intermittent cooling).

[b]Reactions were obtained by exposing the reaction to 1-minute cycling pulse sequences of US combined with IR irradiation (with 5-min intermittent cooling).

[c]Reactions were subjected to one pulse sequences by the required time.

[d]Reactions were submitted to react in presence of 1.8 mol% (1 equiv) of radical inhibitor 1,4-benzoquinone (*Q*).

[e]Reactions were summited to react by the required time.

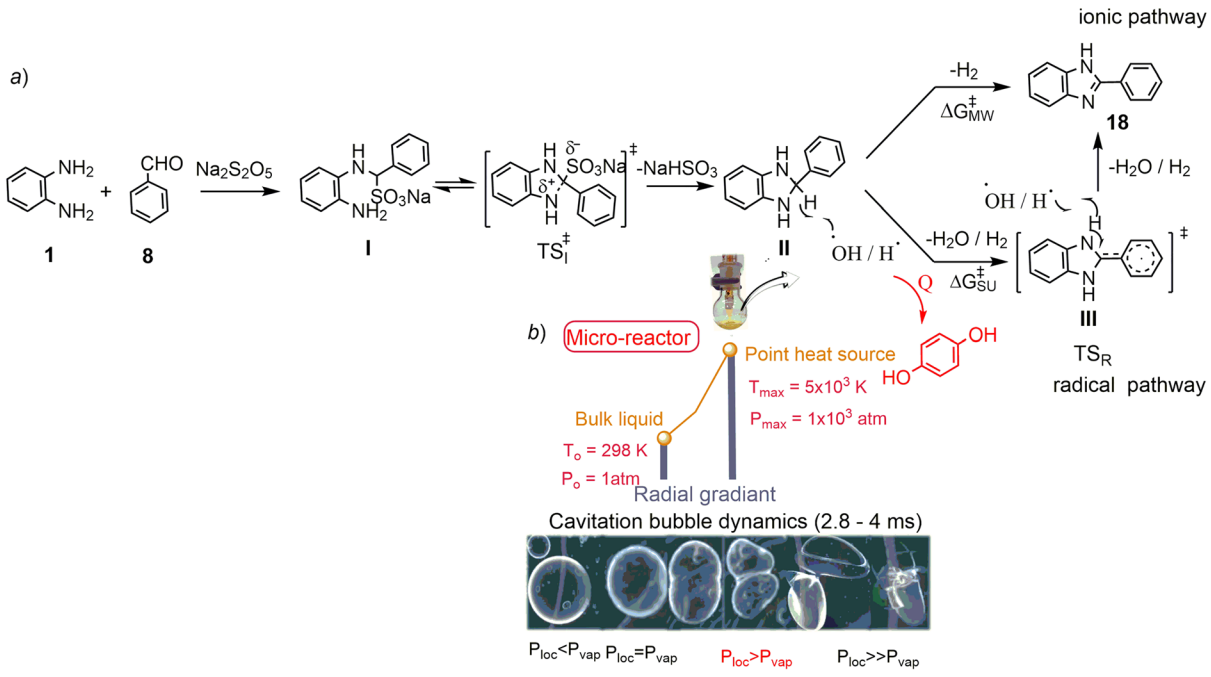


FIGURE 1 (A) Comparison of two feasible pathways of reaction (ionic-radical) induced by MW and SIUI and (B) overview of cavitation bubble dynamics, their physical and chemical effects. In cavitation, bubbles collapse produce transient hot-spots that reach 5000 K and 1000 atm in a short span of time capable to generate a heating/cooling rate of more than 1000 K/s.[17] Thereby, hot-spot drive high-energy chemical reactions predominantly associated with the SET process. SET, single electron transfer

TABLE 2 Comparison of conditions and yields obtained in the preparation of 2,5-disubstituted-1*H*-benzimidazole **19-76**

Entry	Product	Δ[d]	IR[d]	US[a]	SIUI[b]	MW[c]	Mp (°C) Exp./lit.
		Time (h)/yield (%)					
3	**19**	6.0/88	0.83/78	0.58/80	0.16/87	0.016/85	232-234/235-236[15]
4	**20**	5.5/89	0.66/66	1.00/83	0.25/88	0.016/88	287-299/288-290[22]
5	**21**	4.0/78	0.30/87	0.11/80	0.03/77	0.016/88	218-220/219[23]
6	**22**	5.0/88	0.50/92	0.21/68	0.15/93	0.025/89	257-259/258-260[24]
7	**23**	6.0/87	0.46/87	0.18/88	0.03/86	0.021/87	246-247/246-248[25]
8	**24**	6.0/76	1.00/60	0.83/75	0.25/70	0.016/72	233-235/232-235[24]
9	**25**	6.0/84	0.53/82	0.50/80	0.16/76	0.021/82	205-206/204-206[25]
10	**26**	6.0/80	0.80/78	1.00/82	0.33/83	0.023/70	299-301/298-300[25]
11	**27**	3.0/86	0.66/65	0.30/65	0.20/87	0.35/90	186-188[26]
12	**28**	4.0/89	0.83/49	0.28/60	0.15/71	0.30/87	206-208/207-208[27]
13	**29**	2.5/93	0.91/34	0.65/53	0.48/60	0.50/88	169-172/168-171[28]
14	**30**	2.0/83	0.83/37	0.61/61	0.50/59	0.40/87	199-201/198-199[29]
15	**31**	3.0/78	0.66/55	0.63/70	0.48/77	0.50/75	232-234
16	**32**	3.0/86	0.91/36	0.78/48	0.53/50	0.66/73	228-230
17	**33**	3.0/84	0.83/32	0.90/42	0.63/42	0.20/67	285-286/283-285
18	**34**	5.0/76	0.66/36	0.80/69	0.25/59	0.33/74	190-191/190-193[30]
19	**35**	4.0/87	1.00/38	0.71/46	0.60/57	0.46/80	263-265/265-267[31]
20	**36**	5.0/82	1.00/39	0.40/53	0.33/63	0.36/82	224-226/225-228[32]
21	**37**	2.0/98	0.66/45	0.53/55	0.46/67	0.41/70	130-133[33]
22	**38**	2.5/93	0.50/73	0.23/73	0.16/91	0.25/97	290-291[34]
23	**39**	2.0/89	0.41/86	0.33/73	0.21/70	0.16/93	166-169
24	**40**	2.0/80	0.50/85	0.43/61	0.33/69	0.20/94	203-205
25	**41**	2.0/70	0.66/80	0.58/80	0.45/77	0.30/82	178-180
26	**42**	2.0/54	0.50/56	0.33/68	0.31/78	0.21/85	184-186
27	**43**	3.0/58	0.78/57	0.61/72	0.36/72	0.23/83	210-211
28	**44**	3.0/86	0.33/72	0.46/89	0.25/69	0.13/89	223-225
29	**45**	4.0/83	0.25/50	0.55/76	0.38/77	0.10/91	230-231
30	**46**	3.6/83	0.50/60	0.50/78	0.33/83	0.80/90	210-213
31	**47**	04/54	0.75/52	1.00/48	0.66/67	0.41/70	131-133
32	**48**	3.5/64	0.91/65	1.40/60	1.30/65	0.83/58	160-163
33	**49**	4.0/80	1.00/75	1.40/60	1.30/57	0.86/67	122-124
34	**50**	3.0/70	0.91/75	1.30/56	1.20/54	0.90/61	155-157
35	**51**	4.0/54	0.80/52	1.20/48	0.83/67	0.66/70	150-152
36	**52**	3.0/65	0.66/67	1.50/54	1.00/72	0.71/67	165-167
37	**53**	3.0/68	0.75/72	1.00/58	01.1/64	0.75/61	132-134
38	**54**	3.5/30	0.66/65	1.20/60	0.91/65	0.61/58	142-144
39	**55**	3.0/50	0.86/67	0.91/54	1.00/72	0.66/67	145-148
40	**56**	3.5/64	0.78/64	1.20/65	1.30/60	0.50/65	160-162
41	**57**	6.0/88	0.33/65	0.33/70	0.21/75	0.06/76	259-261
42	**58**	3.0/87	0.53/77	1.13/30	0.61/66	0.08/87	197-199
43	**59**	4.5/89	0.50/74	0.83/25	0.33/57	0.13/78	145-147

(Continues)

TABLE 2 (Continued)

Entry	Product	Δ[d]	IR[d]	US[a]	SIUI[b]	MW[c]	Mp (°C) Exp./lit.
44	**60**	4.0/92	0.83/69	1.00/32	0.75/59	0.13/80	210-212
45	**61**	3.0/79	0.45/82	0.70/26	0.50/67	0.25/90	195-197
45	**62**	3.5/73	0.46/87	0.83/22	0.58/70	0.23/86	166-168
47	**63**	4.0/80	0.75/70	1.00/26	0.66/66	0.20/82	133-134
48	**64**	2.0/62	0.53/52	0.86/27	0.75/56	0.30/78	207-209
49	**65**	2.0/47	0.75/47	1.03/24	0.83/47	0.26/62	232-234
50	**66**	1.0/52	0.61/42	1.00/23	1.00/52	0.30/80	245-247
51	**67**	3.0/73	0.91/67	1.00/21	0.46/38	0.15/77	249-251
52	**68**	2.0/80	0.75/77	1.50/27	0.76/47	0.15/68	210-212
53	**69**	6.0/92	0.41/80	0.80/30	0.28/73	0.15/83	268-269
54	**70**	5.5/91	0.58/76	1.00/58	0.25/78	5.50/91	259-261
55	**71**	2.5/82	0.91/88	0.83/29	0.38/61	0.25/79	250-251
56	**72**	2.0/70	1.16/67	1.16/22	0.91/32	0.23/68	234-236
57	**73**	2.5/68	1.00/76	1.50/23	0.83/28	0.30/57	240-242
58	**74**	5.0/82	0.58/78	0.91/38	0.56/56	0.20/86	241-243
59	**75**	4.0/78	0.66/81	0.96/28	0.60/53	0.30/83	226-228
60	**76**	3.5/62	0.63/77	1.00/27	0.66/58	0.20/78	230/229

Note. Reaction condition: *o*-phenylenediamine derivatives (5 mmol), corresponding arenecarbaldehyde (5.5 mmol), $Na_2S_2O_5$ (5 mmol within 5-8 mL), and ethanol 5 mL were subjected to react at 74 ± 2°C.

Abbreviations: IR, infared; MW, microwave; SIUI, simultaneous irradiation with US and IR; US, ultrasound.

[a]Reactions were obtained by exposing the reactants to 2-minute cycling pulse sequences of US (with 5-min intermittent cooling).

[b]Reactions were obtained by exposing the reaction to 1-minute cycling pulse sequences of US combined with IR irradiation (with 5-min intermittent cooling).

[c]Reactions were subjected to one pulse sequences by the required time.

[d]Reactions were summited to react by the required time.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Over the years, several papers have documented the importance of the synergism effect in organic synthesis mainly by a combination of MW with US sources.[13] Notwithstanding, other conjunctiva green techniques such as SIUI has little practical application in the organic chemistry field. Consequently, in an attempt to compare their reliability and efficiency with conventional method (Δ) and two of the most important green activation techniques (viz, MW and the US) in the synthesis of diversely substituted-1*H*-benzimidazoles **18-76**, 4-substituted *o*-phenyendiamine **2-6** were reacted with formaldehyde **7**, and nine arenecaraldehydes derivatives **8-16** in presence of $Na_2S_2O_5$ dissolved in polar media as depicted in Scheme 1.

Data gathering in Table 1 shows that replacement of the aldehyde aliphatic (R^1 = H, entry 1) by aromatic one (R^1 = Ph, entry 2) dramatically reduces reaction times, except for IR method. Nonetheless, this factor seems to impact to a less extent on the efficiency of reaction, which remains without substantial variation. Notably, the assembling of compound **18** at 74 ± 2°C under SIUI method proceeds fivefold faster (90% yield within 1.8 min) than that seen in the formation of **17** (79% yield within 9 min) under similar condition, although the differences of yield do not exceed 11%. Surprisingly, the contrasting effect was found with the MW method, as for this dielectric heating slow down the reaction rate by approximately 1.9 orders of magnitude, despite its evident superior activation capacity. In this regard, the ration rate enhancement of the SIUI-assisted reactions does not exceed approximately 38%; but remarkably, it increases up to 50% (2.5-fold faster) as contrasted with either US or Δ alone. But, more importantly, the reaction exposed to IR irradiation results as effective as the US but reversed in order of magnitude.

On the other hand, Δ/SIUI rate ratio that involves the of use aromatic and aliphatic inputs was deemed in 134:1 (4 h vs 1.8 min) and 67:1 (10 h vs 9 min), respectively. This sizeable increase in the reaction rate acquires even greater significance when the SIUI/MW ratio was compared. In this case, we found that the MW method exceeds the SIUI effect nearly by a factor of 1.9 to 3.

The increased acceleration induced by SIUI was interpreted in terms of excitation of vibrational modes[16] and acoustic cavitation. Particularly, hot-spot and associated chemical effects (such as sonolysis of water: $H_2O \rightarrow HO^{\cdot} + H^{\cdot}$, Figure 1B)[18] that occur during the transient cavitation effect (Figure 1B) might trace alternative pathways of reaction with lowest energy (viz, selectivity changes), eg, mechanistic shifting favored by single electron transfer (SET) process associated with radical transition state ($TS_R^{\ddagger}$) highly stabilized. Various radical intermediates have recently been postulated as key species in the benzimidazole synthesis exposure to visible light irradiation (450 nm) in the presence of molecular oxygen.[19] Accordingly, our explanation is connected to mechanistic pathway changes associated with the postulation of highly stabilized benzyl-type radical species **III** generation, presumably evolving from unstable intermediate benzimidazole **II**[20] prior removal of $H^{\cdot}$ by interaction with radical entities formed during the transient cavitation effect (Figure 1). Succeeding losses of $H^{\cdot}$ from the incipient radical **III** provides the driven force to the formation of the thermodynamically stable benzimidazole product as well as H_2O and H_2 as a byproduct.

To prove the radical intermediate, we have carried out the same reaction under IR, SU, and SIUI in the presence of 1.8 mol% and 1.0 equiv of *Q* as a radical inhibitor. The two latter methods afford a significant decrease in the yield of the corresponding benzimidazole (Table 1, entry 2′), suggesting the possibility of a radical intermediate in the course of the reaction (Figure 1A). On the basis of this observation, we assumed that the stabilization of the postulated $TS_R^{\ddagger}$ pathway should be more effective than the $TS^{\ddagger}_{MW}$ pathway because of the extended conjugation effects inherent to the benzyl-type radical species proposed. Therefore, the presumption is that $G^{\ddagger}_{SIUI} << G^{\ddagger}_{MW}$ supports the enhancement of reactivity by a decrease in the activation energy. Thus, the differences of reactivity observed between SIUI and MW are attributed to that nature and effect of the US are intrinsically different from MW, as for the latter might benefits mostly from highly stabilized ionic transition state ($TS_I^{\ddagger}$) induced by rapid dielectric heating, eg, the tendency of a polarized transition state (or dipole species) to follow the inversion of an alternating electric field.[21] The logical inference from these preliminary findings suggests that aldehydes that fulfill Hückel's (4n + 2) π-electron rule should mostly increase, rather than decrease, the reaction rate of 2-arenebenzimidazole promoted under SIUI condition, as of that the magnitude of the elicited effects depend on the kind of the source of energy used. These results lead to identifying two different relative order of activation depending on the nature of the aromatic and

TABLE 3 Inhibitory activity of compounds **17-76** against *C albicans*, *E coli*, and *S aureous* strains

	Fungus	Bacteria	
		Gram (−)	Gram (+)
Compound	*C albicans* (ATCC 10231)	*E coli* (ATCC 8739)	*S aureus* (ATCC 6538)
17	28.3	21.0	32.0
18	13.0	15.0	21.0
20	—	—	27.0
21	13.0	14.0	28.0
22	9.60	15.0	40.0
24	20.6	30.0	33.0
26	18.3	—	20.0
27	10.3	14.0	18.0
28	8.30	—	28.0
30	7.00	9.00	23.0
31	—	—	22.0
32	—	17.0	23.0
33	7.60	—	18.0
34	8.00	—	22.0
35	10.0	13.0	21.0
37	—	29.0	18.0
38	16.6	31.0	18.0
39	9.30	—	25.0
40	—	—	26.0
41	—	—	27.0
44	10.3	—	23.0
45	—	—	23.0
58	38.0	28.0	18.0
60	—	8.00	20.0
61	14.6	8.00	—
63	42.0	—	—
65	15.6	—	—
66	38.0	13.0	24.0
67	9.30	15.0	33.0
72	—	6.00	—
73	8.00	—	—
74	11.3	13.0	27.0
75	—	7.00	—
76	7.30	—	—
Miconazole	20.0	ND	ND
TMP/SMX	ND	25.0	ND
Ampicillin	ND	ND	30.0

Note. The values of inhibition are expressed in mm.; compounds **19**, **23**, **25**, **29**, **36**, **42**, **43**, **46**-**57**, **59**, **62**, **64**, and **68**-**71** shown none activity.
Abbreviations: *C albicans, Candida albicans*; *E coli*, *Escherichia coli*; *S* aureous, *Staphylococcus aureus*; Not determined.

aliphatic aldehyde used, that is, MW > SIUI > the US > IR > Δ vs MW > SIUI > IR > the US > Δ, respectively.

By contrast, it was found that *p*-monosubstituted phenylenediamines inputs **2**-**3** bearing electron-withdrawing group (EWG, $R = NO_2$,) or electron-donating group (EDG, $R = NH_2$) adversely affect the reaction rate of 5-monosubstituted- and 2,5-disubstituted-1*H*-benzimidazole **27**, **28**, **37**, and **38** when they were subjected to react under nonconventional conditions (Table 2, entries 11, 12, 21, and 22). Thus, when the electron richer 4-aminophenylendiamine **3** reacts with, **7** afford **37** in lower to moderate yield (45-70%, entry 21) despite the longer time of reaction (approximately 25-40 min), wherein the IR effect results to be the least efficient promoter (45% for 40 min, entry 21). Whereas the Δ effect gives excellent yield (98%) with improved times of reaction (2.0 h) as compared with **17**. These contrasting findings are reasonably comparable with the result obtained in the synthesis of **27** (entry 11). Nevertheless, it is evident that the presence of the strong EWG on the phenylendiamine ring mostly improves the yield and rate of reaction of **27** as contrasted with **37**. In this regard, the SIUI effect not only provides an excellent yield (87%) at 74 ± 2°C but also proceeds with a better time of reaction (12 min) that heavily contrasts with Δ, which requires 3 hours of continuous exposure to furnish **27** in similar yield (86%). But more importantly, the MW increases their reaction time 9 minutes more, whereas the US and IR alone lessen the efficiency of the reaction, even with a larger time of reaction. Despite it, the reaction times are reduced from 40 minutes with IR at 74 ± 2°C to 18 minutes with the US, whereas the yields remain unchanged (65%). Note that in the case of Δ, the EDG revert significantly the results, that is, 98% for 2 hours vs 86% for 3 hours as compared with **37** and **27**, respectively.

Highlight that whereas the synthesis of **28** (entry 12) and **27** showed small changes in the rate and yield of the reaction, a noticeable improvement was observed in the preparation of **38** (entry 22), which even resulted better than the preparation of **37**, whatever the nonconventional source of energy used. Consequently, the preparation of **38** by SIUI exposure at 74 ± 2°C for approximately 9.0 minutes furnish 91% of yield versus 71% of the compound **28** at comparable time of reaction, whereas their promotion under Δ affords 93% for 2.5 hours. Highlight that while the latter method requires at least 2.5 to 4 hours of reaction under continuous heating exposure at 74 ± 2°C, the SIUI-assisted reactions were readily accomplished within 10 minutes when phenylendiamine inputs bearing EDG was used. Remarkably, these results differ significantly from the relative order of reactivity observed in the synthesis of **27** and **37** (R^1 = H). But, more importantly, the reaction times of such reactions get improved significantly under the SIUI effect as compared with MW, SU, IR, or Δ. Thus, the order of activation deduced from the different kinds of energies explored appear as SIUI > the US > MW > IR > Δ, which seems to be dependent on the nature of the groups R^1 and *R* installed on the benzimidazole core

In an attempt to assess the role of the electronic effects induced by mono- and disubstituted arenecarbaldehyde **9**-**16** bearing EWG or EDG (R^1 = 4-N(Me)$_2$Ph, 3,4-(OMe)$_2$Ph, and NO_2Ph) in the synthesis of the benzimidazoles derivatives **39**-**46** (Table 2, entries 23-30); further reactions were explored. The experimental results show no appreciable differences upon yields and reaction times when those reactions were exposed to Δ and nonconventional effects at comparable conditions. Besides, 2-phenyl-5-substituted benzimidazoles analogs bearing either phenyldiazenyl (*R* = -N=NPh, **48**), *N*-phenylamino (*R* = -*N*HPh, **58**), or *N*-hydroxymethyl

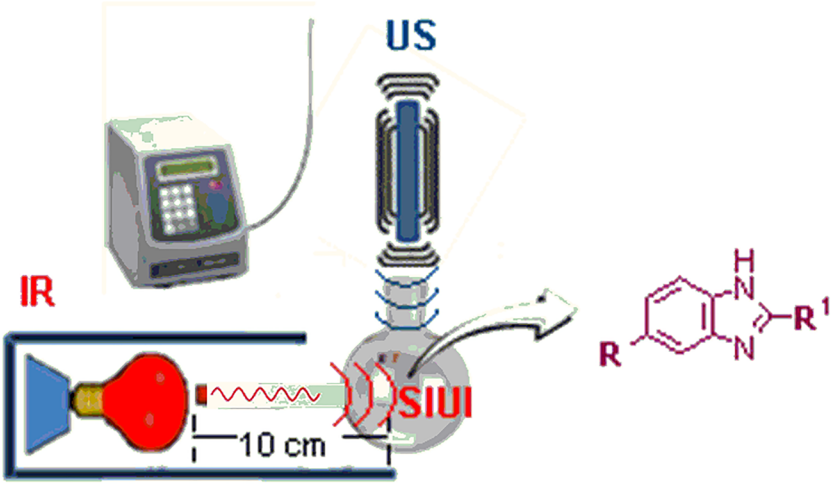


FIGURE 2 Schematic setup of the devices used for the SIUI-assisted organic synthesis. SIUI, simultaneous irradiation with US and IR

amino (R = -NHCH$_2$CH$_2$OH, **68**) moiety at this position generally failed to improve the above parameters as compared with the parent compound **38** (R = NH$_2$). Similar results were also observed in the preparation of compound **49**-**56**, **59**-**66**, and **59**-**66** (Table 2, entries: 33-40, 43-50, and 63-60, respectively). Consequently, the general order of activation deduced from these activating groups, regardless of the approach used appeared as NH$_2$ > NHPh > NHCH$_2$CH$_2$OH > N = NPh (entries 22, 32, 42, and 52). Notably, the MW technology gets improved in terms of time and yield of reaction over the remaining methods. On the basis of this result, it seems that the reaction times depend mostly on the electronic effects induced by the R-group attached to the phenylendiamine core rather than on the nature and pattern of substitution of the phenyl ring installing on C-2 of the imidazole motif.

2.2 | Antifungal and antibacterial evaluation

Antimicrobial and antifungal screening of test compounds **17**-**76** carried out by the disk diffusion test is summarized in Table 3. Unfortunately, most synthesized

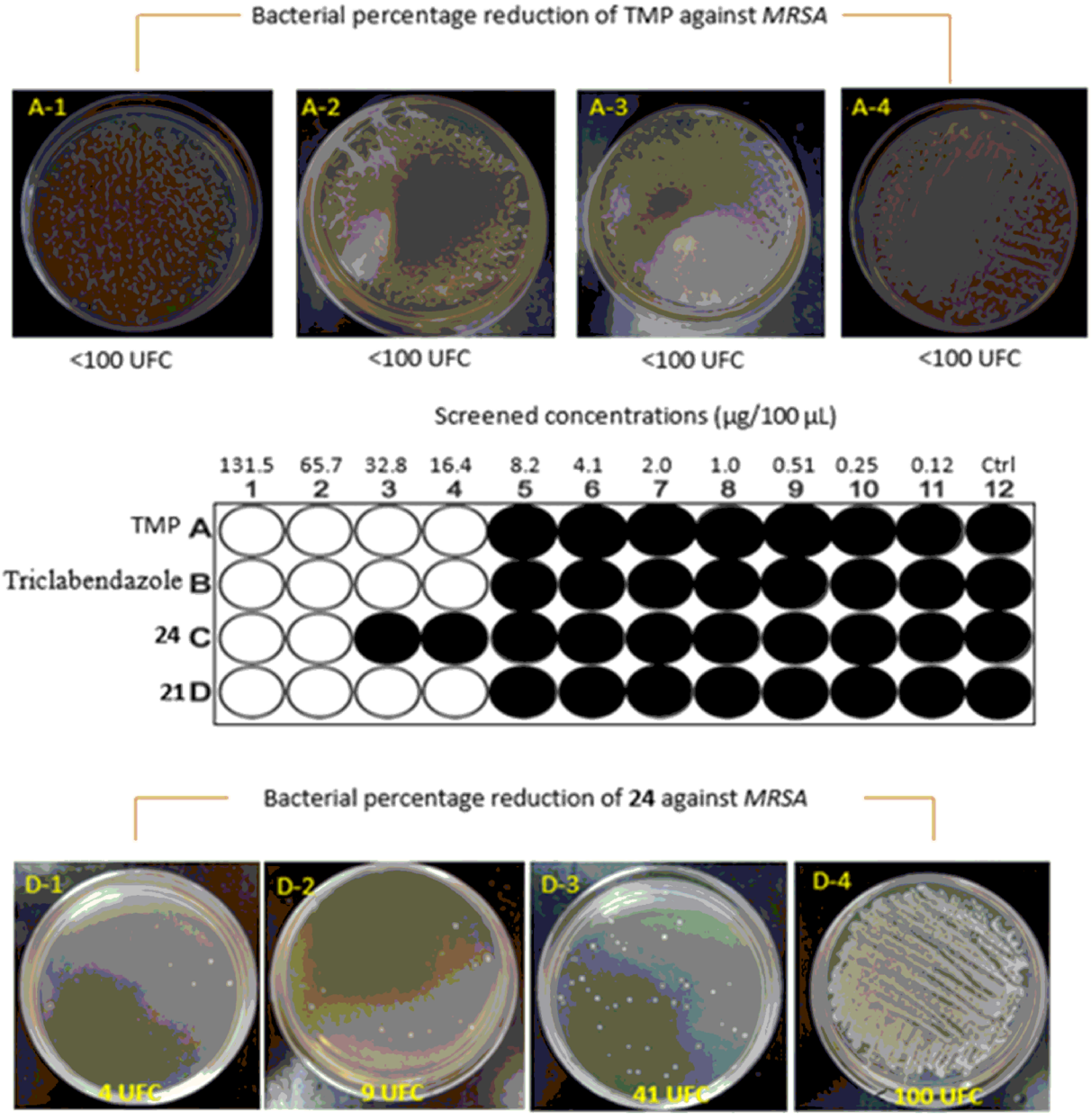


FIGURE 3 MIC values of TMP, triclabendazole, **21** and **24** against MRSA microorganisms (middle) and UFCs observed after re-incubating TMP (A1-A4, up) and 24 (D1-D4, bottom) with MRSA in a concentration of 16.4 µg/100 µL to 131.5 µg/100 µL. MIC, minimum inhibitory concentration

compounds did not show promising activity against wild type *E coli* (ATCC 8739), at best compounds **24**, **37**-**38**, and **58** shown activities comparable with trimethoprim-sulfamethoxazole formulation (TMP/SMX) used as reference drug.

Notably, the unsubstituted benzimidazole **17** exhibits improved antifungal activity than Miconazole at the highest tested concentration (4 mg/100 μL) explored. Whereas compound **63** with 4-aminophenyl and *N*-phenylamino moieties (R^1 = 4-NH_2Ph, R = -NHPh) on C2 and C5 positions improve twice the biological activity. Interestingly, the replacement of the 4-NH_2Ph group with a hydrogen atom, as in compound **57**, induces complete loss of activity. However, significant antifungal activity was observed when Ph substituent was installed on such a position while maintaining the -NHPh moiety (**58**). In this context, replacement of the 4-NH_2 group on the phenyl ring attached to C2 position with other polar groups such as NO_2, OMe, and -N $(Me)_2$ (compounds **66** and **59**-**60**), shows unfavorable effect, even loss of activity. Such results suggested that the hydrophobic nature of the substituent at C2 position and the capacity of their polar group to form additional hydrogen bonding become determinant to the biological activity.

On the other hand, compound **22** with a 3-NH_2Ph group installed on the C2 position showed promising activity against wild type *S aureus*, whereas the compound **25** bearing a more polar 3-NO_2Ph group on this position loses their microbial activity. In this connection, the compounds bearing those polar groups either on C2 or C4 position of the phenyl ring, that is, compounds **21**, **24** and **26**, display decreasing activity, whereas **23** results were inactive. The substantial activity exhibited for **22**, allegedly due to the better capacity of the -NH_2 group for hydrogen bonding, argued for additional SAR development around the 5-positions of the benzimidazole core with polar groups able to form additional hydrogen bonding that could impart even greater hydrophilic capacity. This assumption prompts us to synthesize compound **72** bearing 2-hydroxyethyl amino group on C5 position while maintaining the 3-NH_2Ph moiety on C2 position. However, the presence of this more hydrophilic group gives complete loss of activity. Likewise, the removal of the 3-NH_2 group on the phenyl ring attached to C2 (compound **68**) elicit a lack of biological activity. However, the replacement of 3-NH_2Ph moiety by hydrogen atom while maintaining the 2-hydroxyethyl amino group on the C5 position (compound **67**) shows activity comparable with ampicillin drug.

Even though most of the screened compounds showed good qualitative activity by disk diffusion test, only the compound giving excellent growth inhibition zones was further tested by microdilution assay[35,36] to determine their MIC values. The results show that the MRSA ATCC43300 strain presented high sensibility to the compound **21**, which exhibits a MIC greater than 16.4 μg/100 μL comparable with TMP used as the reference drug (Figure 3). Unfortunately, decreased sensibility was found for compound **24** (MIC ≥ 65.7 μg/100 μL). Notably, none of the screened compounds were active against *E coli* at a concentration range from 0.12 μg/100 μL to 131.5 μg/100 μL; however, TMP shows a MIC greater than 1.02 μg/100 μL. As evidenced by these results, the benzimidazole **21** exhibits a high selectivity toward Gram(+) microorganism and bacterial percentage reduction of 96%, which contrasts with 0.0% of TMP.

3 | CONCLUSION

We demonstrated that combined US/IR irradiation can accelerate significantly the formation of 2-arenesubstituted benzimidazoles depending on the chemical nature of the reactants used. But more importantly, the different nature of the combined tools may impact significantly on the pathway of reaction, which affects the rate and efficiency of the reaction. Although we did not fully understand how the combination of cavitation and IR effect influence the course of the reaction, a process requiring ionic-radical sequential mechanism might benefit from these green methods. Thus, this strategy may be considered a promising and cost-effective new application, but after all, an emerging technological innovation deserves further attention in organic synthesis.

4 | EXPERIMENTAL

4.1 | Materials and general methods

All commercial reagents and solvents were of reagent grade from Sigma-Aldrich and used as received without further purification. Analytical thin layer and preparative chromatography were performed on precoated Kieselgel $60F_{254}$ plates and Gel silice MN-Kieselgel G/UV_{254}, respectively; spots were located using UV (254 nm). All melting points were measured in a MEL-TEMP II Electrothermal melting point apparatus and remain uncorrected. ^{1}H and ^{13}C NMR spectra were obtained either in DMSO-d_6 or $CDCl_3$ using a 300-MHz spectrometer. Chemical shifts (δ) are expressed in parts per million relative to the tetramethylsilane peak used as an internal standard. The *J* values are in hertz, and the splitting patterns are designated as follows: *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet; and *bs*, broad singlet. IR spectra

were recorded on an FTIR Bruker spectrophotometer using attenuated total reflection method. Mass spectra (MS) and high-resolution mass spectra (HRMS) were obtained using a JEOLJMS-AX505 spectrometer.

4.2 | Experimental equipment

4.2.1 | US-IR coupling

The devices used herein is composed of two independent blocks: a Cole Parmer 500-W 20 kHz ± 50 Hz ultrasonic processor and a homemade IR device adapted with a THERA-TERM OSRAM 250-W Red IR bulb, 125 V which emit a wavelength of 1100 nm (9.09 cm^{-1}) and a thermostat to regulate the power output. Both of these devices were assembled as shown in Figure 2. All reactions were submitted to react after locating the IR device at 10 cm away of the reaction flask while holding the ultrasonic horn at approximately 1.4 cm within the reactant system with the US equipment working at 80% of potency and 50% of amplitude at an irradiation frequency of 20 kHz ± 50 Hz, while the reaction temperature was monitored with an InfraPro IR thermometer.

4.2.2 | Ultrasound

A Cole Parmer 500-W 20 kHz ± 50 Hz ultrasonic processor, 120 VAC was used in the standard configuration as delivered, and the reaction temperature was monitored with an InfraPro IR thermometer.

4.2.3 | Infrared

An electric homemade metallic cylindrical can (29.5 cm in length with a diameter of 15.3 cm of width) designed to fix and adjust the position of an IR emission bulb was assembled with a THERA-TERM OSRAM 250-W Red IR bulb, 125 V which emits a wavelength of 1100 nm (9.09 cm^{-1}). The device is adapted with a varicap diode to regulating the power output. The reaction temperature was monitored with an InfraPro IR thermometer.

4.2.4 | Microwave

A monowave of 300 MW synthesis reactor by Anton Paar was used in the standard configuration as delivered. All experiments we carry out in a sealed MW vial (20 mL) and the reaction temperature were monitored with an immersing ruby thermometer.

4.3 | General procedure for the synthesis of 1*H*-benzimidazole derivatives (17-76)

4.3.1 | MW reactions

A freshly prepared aqueous solution of $Na_2S_2O_5$ (5 mmol, 0.95 g, 5-8 mL) was added to a stirring solution of *o*-phenylenediamine derivate **1-6** (5 mmol, 0.554 g) and the corresponding aldehyde **7-16** (5.5 mmol) in ethanol (5 mL). The mixture was transferred to a 20-mL vial, sealed and exposed to MW irradiation in monowave of 300 MW of reactors, Anton Paar at 850 W of power in the required time (refer to Tables 1 and 2) at 74 ± 2°C. The progress of reactions was monitored by TLC. After the crude of the reaction was cooled, it was poured onto stirred ice water. The precipitate was collected, dried, and purified by preparative chromatography using an eluting system of hexane/ethyl acetate (7:3). The scraping spot was extracted with acetone and dried to obtain the spectroscopically pure compounds **17-76**.

4.3.2 | Thermic reactions

A 25-mL flask charged with a stirring bar and the corresponding reactant mixture prepared as previously described was submitted to react in the required time (refer to Tables 1 and 2) at 74 ± 2°C using an ordinary chemical heating mantle. Afterward, isolation and purification were carried out as described in Section 4.3.1.

4.3.3 | IR reactions

The corresponding reactant mixture prepared as in Section 4.3.1 was exposed to IR irradiation using a THERA-TERM OSRAM 250-W Red IR bulb in the required time (refer to Tables 1 and 2) at 74 ± 2°C. All reactions were promoted by placing the IR bulb at approximately 10 cm away from the reaction flask. The resulting crude of the reaction was processed according to Section 4.3.1.

Ultrasonic reactions

Method A. The corresponding reactant mixture prepared as before was exposed to a Cole Parmer 500-W 20 KHz ± 50 Hz ultrasonic processor in the required time (refer to Tables 1 and 2) at 74 ± 2°C. The reactions were obtained by exposing the mixture to 2-minute cycling pulse sequences with 5-minute intermittent cooling, while holding the ultrasonic horn at 1.4 cm within the reactant system with the

US equipment working at 80% of potency and 50% of amplitude at an irradiation frequency of 20 kHz ± 50 Hz. The resulting crude of the reaction was processed as described previously.

Method B. (synthesis of **18** in the presence of a radical inhibitor agent). Following method A, the corresponding mixture of the reaction was subjected to react in the presence of 1.8 mol% and 1 equiv of 1,4-benzoquinone to afford 67% and 37% of yield. The resulting crude of the reaction was processed as described previously.

4.3.4 | US-IR coupling

The corresponding reactant mixture was exposed simultaneously to a Cole Parmer 500-W 20 kHz ± 50 Hz ultrasonic processor and a homemade IR devise in the required time (refer to Tables 1 and 2) at 74 ± 2°C. The reactions were summited to react by exposing the reaction to repetitive 1-minute cycling pulse sequences of US combined with IR irradiation (with 5-min intermittent cooling). Afterward, the compounds were isolated and purified as described in the MW section.

The known products were identified by comparing their spectral parameters and physical constants with those reported in the literature, and the new ones were fully characterized using spectrometric and spectroscopic methods. Only physical data of some selected 1*H*-benzimidazoles are included here, for comprehensive information refer to Data S1.

*1H-Benzimidazole (**17**)*

White crystals; yield: 90%; [lit. 14]; mp: 170°C to 171°C; IR (ATR): ν_{max} in cm^{-1}: 1770 (CN), 1477 (C═C), 3113 (NH), 3038, 3061, 3000, 2942, 2862, 2792, 2540 (CH's). ^1H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.19 (q, J = 6.0 and J = 3.0 Hz, 2H), 7.62 (q, J = 6.0 and J = 3.3 Hz, 2H), 8.27 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 115.7, 122.2, 138.5, 142.4 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_7H_7N_2$: 119.06092, found: 119.06055.

*2-Phenyl-1H-benzimidazole (**18**)*

White powder; yield: 96%; [lit. 15]; mp: 290°C to 291°C; IR (ATR): ν_{max} in cm^{-1}: 3372 (NH), 3046, 2961, 2920, 2853, 2750, 2716, 2672, 2584 (CH's), 1588 (CN), 1441 (C═C). ^1H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.24 (d, J = 6 Hz, 2H), 7.48-7.61 (m, 4H), 7.71 (d, J = 6 Hz, 1H), 8.22 (dd, J = 6 and J = 3 Hz, 2H), 12.98 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 111.8, 119.3, 122.1, 123.0, 126.9, 129.4, 130.3, 130.6, 151.7 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{13}H_{11}N_2$: 195.09222, found: 195.09209.

*2-(4-N,N-Dimethylaminophenyl)-1H-benzimidazole (**19**)*

White powder; yield: 89%; [lit. 16]; mp: 287°C to 299°C; IR (ATR): ν_{max} in cm^{-1}: 3421, 3279 (NH), 3051, 2921, 2805, 2666 (CH's CH_3), 1609 (CN), 1469 (C═C). ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$/TMS) δ: 3.00 (s, 3H), 4.38 (s, 1H, NH), 6.67 (s, J = 9.0 Hz, 2H), 7.18 (q, J = 5.7 and J = 3.3 Hz, 2H), 7.55 (q, J = 6.0 and J = 3.0 Hz, 2H), 7.89 (d, J = 8.7 Hz, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6 + $CDCl_3$/TMS) δ: 39.5, 111.9, 117.5, 121.5, 124.7, 127.7, 129.4, 151.3, 152.4 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. For $C_{15}H_{15}N_3$: 238.13442, found: 238.13449.

*2-(3,4-Dimethoxyphenyl)-1H-benzimidazole (**20**)*

White powder; yield: 88%; [lit. 17]; mp: 232°C to 234°C; IR (ATR): ν_{max} in cm^{-1}: 3112, 3100 (NH), 3053, 2974, 2939, 2886, 2845, 2797 (CH's, CH_3), 1604 (CN), 1443 (C═C), 1020 (C═O). ^1H NMR (300 MHz; DMSO-d_6/TMS) δ: 3.63 (s, 3H), 3.67 (s, 1H, NH), 6.7 (d, J = 8.4 Hz, 1H), 6.95-7.01 (m, 2H), 7.35-7.40 (m, 2H), 7.54-7.58 (m, 1H), 7.61 (t, J = 9.0 Hz, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 39.1, 39.4, 109.5, 110.5, 114.1, 119.4, 121.5, 121.9, 137.9, 148.5, 150.1, 151.2 ppm. DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{15}H_{15}N_2O_2$: 255.11335, found: 255.11347.

*2-(2-Aminophenyl)-1H-benzimidazole (**21**)*

Gray light powder; yield: 88%; [lit. 18]; mp: 218°C to 220°C; IR (ATR): ν_{max} in cm^{-1}: 3429, 3376 (NH), 3325, 3209 (NH), 3050, 3045 (CH's), 1632 (CN), 1464 (C═C), 761, 713, 646. ^1H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.66 (s, 3H, NH), 6.68 (t, J = 7.5 Hz, 1H), 7.09 (t, J = 7.5 Hz, 1H), 7.48 (t, J = 7.2 Hz, 1H), 7.59-7.62 (m, 2H), 7.71 (t, J = 7.8 Hz, 2H), 7.80 (d, J = 7.8 Hz, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 110.5, 114.9, 115.5, 116.6, 122.4, 127.8, 130.9, 138.6, 148.7, 153.0 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{13}H_{12}N_3$, 210.10312; found: 210.10258.

*2-(3-Aminophenyl)-1H-benzimidazole (**22**)*

Gray light powder; yield: 93%; [lit. 19]; mp: 257°C to 259°C; IR (ATR): ν_{max} in cm^{-1}: 3184 (NH), 3058 (CH's), 1611 (CN), 1443 (C═C). ^1H NMR (300 MHz, DMSO-d_6/TMS) δ: 4.09 (s, 3H), 6.76 (d, J = 7.8 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 8.32 (d, J = 2.1 Hz, 1H), 8.35 (d, J = 1.8 Hz, 1H), 8.43 (d, J = 7.8 Hz, 1H), 8.51 (d, J = 7.8 Hz, 1H), 8.62 (d, J = 7.8 Hz, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 111.2, 112.4, 118. 0, 119.2, 121.0, 121.3, 131.2, 132.9, 144.9, 148.8, 153.1 ppm. HR-

DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{13}H_{12}N_3$: 210.10312, found: 210.10343.

*2-(4-Aminophenyl)-1H-benzimidazole (**23**)*

Gray light powder; Yield: 88%; [lit. 20]; mp: 246°C to 247°C; IR (ATR): ν_{max} in cm^{-1}: 3291 (NH), 3068, 2825 (CH's), 1681 (CN), 1452 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 6.73 (d, J = 9.0 Hz, 2H), 7.22 (d, J = 9.0 Hz, 2H), 7.32 (m, 3H, NH_2, NH), 7.85 (d, J = 9.0 Hz, 2H), 7.93 (d, J = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 123.4, 126.3, 127.4, 127.6, 131.5, 144.7, 150.0, 150.7 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{13}H_{12}N_3$: 210.10312, found: 210.10330.

*2-(2-Nitrophenyl)-1H-benzimidazole (**24**)*

Yellow light powder; yield: 76%; [lit. 19]; mp: 233°C to 235°C; IR (ATR): ν_{max} in cm^{-1}: 3535, 3476 (NH), 3440, 3239 (NH), 3108, 2850 (CH's), 1601 (CN), 1517, 1344 (NO_2), 1454 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.25 (dd, J = 5.4 and J = 2.4 Hz, 2H) 7.63 (s, 2H), 7.73 (t, J = 7.7 Hz, 1H), 7.85 (t, J = 7.6 Hz, 1H), 8.01 (dd, J = 7.8 and J = 2.6 Hz, 2H), 13.10 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 112.6, 119.3, 123.0, 124.7, 131.3, 131.4, 133.1, 135.7, 143.9, 147.8, 149.4 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{13}H_{10}N_3O_2$: 240.08132, found: 240.08043.

*2-(3-Nitrophenyl)-1H-benzimidazole (**25**)*

Yellow light powder; yield: 84%; [lit. 20]; mp: 205°C to 206°C; IR (ATR): ν_{max} in cm^{-1}: 3208, 3105 (NH), 2992, 2856 (CH's), 1621 (CN), 1520, 1348 (NO_2), 1475 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6 + $CDCl_3$/TMS) δ: 2.60 (s, 1H, NH), 7.27 (q, J = 6.0 and J = 3.3 Hz, 2H), 7.66 (t, J = 8.1 Hz, 1H), 8.26 (dq, J = 5.7 and J = 1.8 and J = 0.9 Hz, 1H), 8.27 (dq, J = 5.7 and J = 1.8 and J = 0.9 Hz, 1H), 8.63 (t, J = 1.2 Hz, 1H), 8.66 (t, J = 1.2 Hz, 1H), 9.08 (t, J = 2.1 Hz, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6 + $CDCl_3$/TMS) δ: 120.8, 122.3, 123.4, 129.4, 131.7, 132.2, 148.0, 148.9 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{13}H_{10}N_3O_2$: 240.08132, found: 240.08133.

*2-(4-Nitrophenyl)-1H-benzimidazole (**26**)*

Yellow light powder; yield: 83%; [lit. 20]; mp: 299°C to 301°C; IR (ATR): ν_{max} in cm^{-1}: 3442 (NH), 2922, 2853, 2744 (CH's), 1603 (CN), 1513, 1339 (NO_2), 1433 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6 + $CDCl_3$/TMS) δ: 2.92 (s, 1H, NH), 7.29 (q, J = 6.7 and J = 3.3 Hz, 2H), 7.67 (q, J = 5.7 and J = 3.0 Hz, 2H), 8.31 (t, J = 2.1 Hz, 1H), 8.34 (t, J = 2.1 Hz, 1H), 8.41 (t, J = 2.1 Hz, 1H), 8.44 (t, J = 1.8 Hz, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6 + $CDCl_3$/TMS) δ: 122.7, 123.6, 127.0, 136.5, 148.2, 149.5, 164.8 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{13}H_{10}N_3O_2$: 240.08132, found: 240.08071.

*5(6)-Nitro-1H-benzimidazole (**27**)*

Yellow light powder; yield: 98%; [lit. 21]; mp: 202°C to204°C; IR (ATR): ν_{max} in cm^{-1}: 3100 (NH), 2918, 2851, 2813, 2723 (CH's), 1623, 1590 (CN), 1513, 1339 (NO_2), 1457 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.76 (d, J = 9.3 Hz, 1H), 8.10 (q, J = 2.4 and 9.3 Hz, 1H), 8.50 (d, J = 2.1 Hz, 1H), 8.55 (s, 1H), 13.09 (sb, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 112.7, 114.8, 117.5, 138.5, 142.6, 146.7 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_7H_6N_3O_2$: 164.04600, found: 164.04612.

*5(6)-Nitro-2-phenyl-1H-benzimidazole (**28**)*

Red light powder; yield: 87%; [lit. 22]; mp: 208°C to 210°C; IR (ATR): ν_{max} in cm^{-1}: 3282 (NH), 3055, 2994, 2924, 2858, 2803, 2729 (CH's), 1628, 1597 (CN), 1521, 1338 (NO_2), 1474 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.62 (d, J = 6.6 Hz, 3H), 7.78 (s, 1H), 8.23-8.14 (m, 3H), 8.51 (s, 1H), 13.64 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 118.44, 127.46, 129.51, 129.62, 131.43, 143.18 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{13}H_{10}N_3O_2$: 240.07730, found: 240.07664.

*5(6)-Nitro-2-(4-N,N-dimethylaminophenyl)-1H-benzimidazole (**29**)*

Red light powder; yield: 88%; [lit. 23]; mp: 170°C to 172°C; IR (ATR): ν_{max} in cm^{-1}: 3434, 3378 (NHas), 3340, 3292, 3223 (NHsim), 3100, 3078, 2900 (CH's), 1638, 1606 (CN), 1520, 1284 (NO_2), 1468 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.05 (s, 6H, $2CH_3$), 6.90 (d, J = 6.9 Hz, 2H), 7.75 (d, J = 9.0 Hz, 1H), 8.07 (d, J = 9.0 Hz, 2H), 8.17 (dd, J = 9.0 and J = 3.0 Hz, 1H), 8.38 (d, J = 3.0 Hz, 1H), 8.98 (sbb, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 46.7, 112.2, 112.8, 119.2, 122.0, 129.2, 139.4, 146.9, 152.8, 158.1 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{15}H_{15}N_4O_2$: 283.11950, found: 283.11976.

*5(6)-Nitro-2-(3,4-dimethoxyphenyl)-1H-benzimidazole (**30**)*

Red light powder; yield: 87%; [lit. 24]; mp: 229°C to 231°C; IR (ATR): ν_{max} in cm^{-1}: 3227 (NH), 2934, 2837 (CH's), 1639, 1600 (CN), 1502, 1264 (NO_2), 1460 (C═C), 1065, 1022 (C—O). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.85 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 7.15 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 6.0 Hz, 1H), 7.77 (s, 1H), 7.80 (s, 1H), 8.09 (d, J = 9.0 Hz, 1H), 8.42 (s, 1H), 15.43 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 46.1, 46.4, 105.1, 111.3, 112.3, 115.9, 129.1, 134.5, 137.3, 144.5, 148.5, 149.2, 150.9, 153.0 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{15}H_{14}N_3O_4$: 300.09843, found: 300.09907.

*5(6)-Nitro 2-(2-aminophenyl)-1H-benzimidazole (**31**)*

Orange light powder; yield: 67%; mp: 285°C to 286°C; IR (ATR): ν_{max} in cm^{-1}: 3434, 3379, 3338, 3293 (NH), 3099, 3077 (CH's), 1619, 1594 (CN), 1520, 1290 (NO_2), 1468 (C═C), 809, 746 (sust 1,2,4), 879 (sust 1,4). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.92 (sb, 2H, NH_2), 6.52 (d, J = 8.4 Hz, 1H), 6.68 (t, J = 7.8 Hz, 1H), 6.87 (d, J = 7.8 Hz, 1H), 7.22 (t, J = 2.1 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 8.13 (q, J = 2.1 and 9.0 Hz, 1H), 8.44 (s, 1H), 12.51 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 109.3, 113.2, 116.3, 117.3, 118.8, 119.4, 126.0, 130.5, 139.8, 142.3, 145.8, 149.5, 154.8 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{11}N_4O_2$: 255.08820, found: 255.08809.

*5(6)-Nitro 2-(3-aminophenyl)-1H-benzimidazole (**32**)*

Orange light powder; yield: 73%; mp: 228°C to 230°C; IR (ATR): ν_{max} in cm^{-1}: 3507, 3362 (NH), 3097, 2919, 2880 (CH's), 1629, 1592 (CN), 1521, 1339 (NO_2), 1475 (C═C), 818, 739 (sust 1,2,4), 843, 739, 689 (sust 1,3). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 4.38 (sb, 2H, NH_2), 6.76 (d, J = 8.1 Hz, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 8.15 (d, J = 8.7 Hz, 1H), 8.30-8.40 (m, 1H), 8.62 (d, J = 7.8 Hz, 1H), 9.01 (d, J = 1.5 Hz, 1H), 13.94 (sb, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 111.6, 114.6, 1157, 116.5, 117.9, 121.6, 129.3, 136.9, 143.4, 147.9, 149.2, 158.8 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{11}N_4O_2$: 255.08820, found: 255.08756.

*5(6)-Nitro 2-(4-aminophenyl)-1H-benzimidazole (**33**)*

Orange light powder; yield: 77%; mp: 232°C to 234°C; IR (ATR): ν_{max} in cm^{-1}: 3597, 3343, 3211 (NH), 3108 (CH's), 1626, 1598 (CN), 1496, 1337 (NO_2), 1411 (C═C), 858, 726 (sust 1,2,4), 818 (sust 1,4). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.05 (s, 2H, NH2), 6.53 (d, J = 9 Hz, 2H), 6.80 (d, J = 9 Hz, 1H), 7.37-7.42 (m, 3H), 7.96 (d, J = 9 Hz), 13.24 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 111.8, 113.7 113.8 116.0, 124.2 135.0 136.3 142.1 143.7 157.9 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{11}N_4O_2$: 255.08820, found: 255.08779.

*5(6)-Nitro-2-(2-nitrophenyl)-1H-benzimidazole (**34**)*

Red light powder; yield: 86%; [lit. 25]; mp: 129°C to 131°C; IR (ATR): ν_{max} in cm^{-1}: 3553 (NH), 3098, 2922, 2859, 2800 (CH's), 1625, 1593, (CN), 1528, 1339 (NO_2), 1432 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.49-7.63 (m, 2H), 7.70-7.84 (m, 2H), 7.90-8.00 (m, 3H), 12.60 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 116.5, 117.4, 119.6, 124.7, 127.1, 128.8, 133.2, 135.2, 139.5, 143.7, 147.9, 149.0, 152.1 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_8N_4O_4$: 285.06238, found: 285.06113.

*5(6)-Nitro-2-(3-nitrophenyl)-1H-benzimidazole (**35**)*

Red light powder; yield: 87%; [lit. 26]; mp: 282°C to 284°C; IR (ATR): ν_{max} in cm^{-1}: 3495, 3437, 3380, 3223 (NH), 3101, 3076 (CH's), 1626, 1606 (CN), 1519, 1346 (NO_2), 1431 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 6.92-7.16 (m, 3H), 784 (d, J = 8.0 Hz, 1H), 8.04-8.15 (m, 1H), 8.72 (d, J = 7.8 Hz, 2H), 11.92 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 111.0, 117.4, 118.0, 122.6, 123.2, 129.5, 129.7, 130.2, 139.0, 144.6, 149.6, 150.1, 150.2 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_9N_4O_4$: 285.06238, found: 285.06111.

*5(6)-Nitro-2-(4-nitrophenyl)-1H-benzimidazole (**36**)*

Red light powder; yield: 82%; [lit. 27]; mp: 298°C to 300°C; IR (ATR): ν_{max} in cm^{-1}: 3338, 3221 (NH), 3099, 2864 (CH's), 1625, 1605 (CN), 1507, 1337 (NO_2), 1469 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.32 (d, J = 8.7 Hz, 1H), 7.43 (s, 1H), 7.66-7.71 (m, 3H), 8.17 (d, J = 7.5 Hz, 2H), 12.02 (sb, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 112.1, 115.1, 117.9, 124.7, 128.8. 134.4, 140.4, 144.0, 147.0, 147.7, 152.9 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_9N_4O_4$: 285.062381, found: 285.06220.

*5(6)-Amino-1H-benzimidazole (**37**)*

White powder; Yield: 90%; [lit. 28]; mp: 107°C to 109°C; IR (ATR): ν_{max} in cm^{-1}: 3276, 3151 (NH), 2930, 2898 (CH's), 1635 (CN), 1506 (C═C). ^{1}H NMR 300 MHz, DMSO-d_6/TMS) δ: 5.20 (sb, 2H, NH_2), 6.51 (d, J = 3.0 Hz, 1H), 6.67 (s, 1H), 7.25 (d, J = 6.0 Hz, 1H), 7.86 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 97.0, 111.7, 117.3, 133.2, 137.2, 139.8, 144.8 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_7H_8N_3$: 134.07182, found: 134.07281.

*5(6)-Amino-2-phenyl-1H-benzimidazole (**38**)*

White powder; yield: 97%; [lit. 29]; mp: 190°C to 192°C; IR (ATR): ν_{max} in cm^{-1}: 3421, 3263 (NH), 3066, 2949, 2782 (CH's), 1641 (CN), 1477 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.02 (s, 1H, NH_2), 6.86 (q, J = 9.0 and 3.0 Hz, 1H), 7.11 (d, J = 3.0 Hz, 1H), 7.57-7.60 (m, 4H), 8.15 (q, J = 6.0 and 3.0 Hz, 2H), 12.47 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 104.7, 110.7, 113.8, 126.7, 127.0, 129.2, 131.6, 142.4, 144.0, 155.0, 162.6 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{12}N_3$: 210.10312, found: 210.10372.

*5(6)-Amino-2-(4-N,N-dimethylaminophenyl)-1H-benzimidazole (**39**)*

White powder; yield: 93%; mp: 260°C to 264°C; IR (ATR): ν_{max} in cm^{-1}: 3251 (NH), 3072, 2935, 611 (CH's, CH_3), 1634 (CN), 1607(C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.94 (sb, 2H, NH_2), 4.18 (s, 6H, $2CH_3$), 6.32 (s,

2H), 6.53 (s, 1H), 6.66 (d, J = 7.8 Hz, 1H), 6.97 (d, J = 7.5, 1H), 7.31 (d, J = 8.4 Hz, 2H), 11.08 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 56.2, 96.1, 108.8, 113.7, 113.8, 114.4, 123.7, 129.0, 133.2, 146.1, 148.0, 153.4 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{15}H_{17}N_4$: 253.14085, found: 253.12518.

*5(6)-Amino-2-(3,4-dimethoxyphenyl)-1H-benzimidazole (**40**)*

White powder; yield: 94%; mp: 203°C to 206°C; IR (ATR): ν_{max} in cm^{-1}: 3238 (NH), 3064, 2929, 2837 (CH's, CH_3), 1665 (CN), 1509 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.50(s, 3H, CH_3), 3.98 (s, 3H, CH_3), 5.61 (sb, 2H, NH_2), 6.82 (d, J = 7.5 Hz,1H), 7.32 (d, J = 8.4 Hz, 1H), 7.42 (s, 1H), 7.57 (s, 1H), 7.75 (d, J = 9.0 Hz, 2H), 8.17 (d, J = 7.5 Hz, 1H), 10.81 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 56.0, 95.6, 111.6, 112.5, 115.6, 119.2, 123.5, 134.6, 140.2, 143.2, 148.0, 153.2 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{15}H_{16}N_3O_2$: 270.12425, found: 270.12413.

*5(6)-Amino-2-(2-aminophenyl)-1H-benzimidazole (**41**)*

Gray light powder; yield: 82%; mp: 278°C to 280°C; IR (ATR): ν_{max} in cm^{-1}: 3344, 3240 (NH), 3063, 2976, 2933 (CH's), 1622 (CN), 1591 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.05 (s, 2H, NH_2), 6.0.3 (s, 2H, NH_2), 7.48 (d, J = 7.2 Hz, 1H), 7.75 (t, J = 7.8 Hz, 1H), 7.85 (t, J = 7.5 Hz, 1H), 7.98 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 8.43 (d, J = 7.5 Hz, 1H, 11.04 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 99.1, 108.4, 11.8, 113.7, 113.8, 116.0, 125.5, 130.5, 134.3, 136.3, 142.1, 143.7, 157.9 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{13}N_4$: 225.11402, found: 225.11385.

*5(6)-Amino-2-(3-aminophenyl)-1H-benzimidazole (**42**)*

Gray powder; yield: 86%; mp: 284°C to 286°C; IR (ATR): ν_{max} in cm^{-1}: 3397, 3222, 3218 (NH), 3131, 2995, 2893 (CH's), 1639 (CN), 1610 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.06 (s, 2H, NH_2), 6.03 (s, 2H, NH_2), 6.52 (d, J = 8.4 Hz, 1H), 6.78 (d, J = 9.0 Hz 1H), 6.90 (s, 1H), 7.38–7.41 (t, 2H), 7.94 (d, J = 9.0 Hz, 2H), 8.34 (d,J = 8.1 Hz, 1H), 10.94 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 96.1, 112.3, 114.8, 116.3, 117.5, 118.2, 124.3, 127.1, 130.0, 131.0, 133.1, 139.5, 143.2, 148.1, 154.4 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{13}N_4$: 225.11402, found: 225.11471.

*5(6)-Amino-2-(4-aminophenyl)-1H-benzimidazole (**43**)*

Gray powder; yield: 84%; mp: >350°C; IR (ATR): ν_{max} in cm^{-1}: 3454, 3330, 3224 (NH), 3059, 2875, 2777 (CH's), 1640 (CN), 1606 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.46 (sb, 4H, $2NH_2$), 6.72-6.78 (m, 3H), 6.84 (d, J = 1.8 Hz, 1H), 7.19-7.21 (m, 2H), 7.38 (d, J = 8.4 Hz, 2H), 10.22 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 97.5, 112.0, 115.1, 116.0, 116.1, 128.2, 130.0, 131.0, 135.1, 139.7, 144.3, 145.1, 152.4 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{13}N_4$: 225.11402, found: 225.11447.

*5(6)-Amino-2-(2-nitrophenyl)-1H-benzimidazole (**44**)*

Yellow powder; yield: 78%; mp: 210°C to 213°C; IR (ATR): ν_{max} in cm^{-1}: 3281, 3198 (NH), 3062 (CH's), 1644 (CN), 1601 (C═C), 1524, 1344 (NO_2), 693, 615 (sust 1,2,4), 800 (sust 1,2). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.77 (s, 2H, NH_2), 6.56 (d, 1H, J = 9.0 Hz), 6.57 (s, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.73-7.78 (t, 1H), 7.95-8.02 (m, 1H), 8.07-8.13 (m, 2H), 11.52 (sb, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 98.1, 110.2, 117.4, 121.7, 122.3, 130.3, 132.2, 133.7, 134.2, 137.2, 145.6, 148.2, 148.8 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{11}N_4O_2$: 255.08820, found: 255.08797.

*5(6)-Amino-2-(3-nitrophenyl)-1H-benzimidazole (**45**)*

Yellow powder; yield: 76%; mp: 230°C to 231°C; IR (ATR): ν_{max} in cm^{-1}: 3210 (NH), 3063 (CH's), 1645 (CN), 1602 (C═C), 1520, 1347 (NO_2), 703, 614 (sust 1,2,4), 860, 801, 699 (sust. 1,3). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.29 (sb, 2H, NH_2), 6.68 (t, J = 7.5 Hz, 1H), 6.88 (d, J = 8.1 Hz, 1H), 7.15-7.24 (m, 3H), 7.60-7.63 (m, 2H), 7.88 (d, J = 7.8 Hz, 1H), 13.87 (sb, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 98.2, 98.5, 110.5, 115.5, 116.6, 122.4, 127.8, 130.9, 132.7, 135.0, 138.6, 144.0, 148.7, 153.0 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{11}N_4O_2$: 255.08820, found: 255.08822.

*5(6)-Amino-2-(4-nitrophenyl)-1H-benzimidazole (**46**)*

Yellow powder; yield: 72%; mp: 223°C to 225°C; IR (ATR): ν_{max} in cm^{-1}: 3454, 3330, 3224 (NH), 3059, 2875, 2777 (CH's), 1640 (CN), 1606 (C═C), 695, 610 (sust 1,2,4), 801 (sust. 1,4). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.55 (sb, 2H, NH_2), 7.13 (d, J = 9.3 Hz, 1H), 7.79 (d, J = 6.9 Hz, 1H), 7.93 (d, J = 6.9 Hz, 1H), 8.01 (s, 2H), 8.50 (d, J = 7.2 Hz, 2H), 11.08 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 99.5, 111.8, 120.6, 122.6, 129.6, 135.7, 141.8, 145.4, 148.6, 152.2 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{13}H_{11}N_4O_2$: 255.08820, found: 255.08834.

*5(6)-(E)-(Phenylazo)-1H-benzimidazole (**47**)*

Violet powder; yield: 47%; mp: 245°C to 248°C; IR (ATR): ν_{max} in cm^{-1}: 3404, 3281 (NH), 3165 (CH's), 1687, 1635 (CN), 1544, 1406 (N═N), 1482 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 6.89 (s, 1H), 7.07 (s, 3H), 7.23 (s, 4H), 8.03 (s, 1H), 10.96 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 113.23, 115.4, 117.9, 112.5, 125.4, 129.5, 129.9, 132.6, 139.2, 142.3, 142.9 ppm.

HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{13}H_{11}N_4$: 223.09837, found: 223.09695.

*5(6)-(E)-(Phenylazo)-2-phenyl-1H-benzimidazole (**48**)*

Violet powder; yield: 52%; mp: 160°C to 162°C; IR (ATR): ν_{max} in cm^{-1}: 3335 (NH), 3198, 3062 (CH's), 1639, 1629 (CN), 1495 (N═N), 1400 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.60-7.97 (m, 13H), 9.79 (1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 109.7, 112.6, 114.5, 115.3, 125.4, 127.4, 128.3, 128.5, 129.8, 134.2, 138.5, 142.9, 146.5, 153.8, 154.4 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{19}H_{15}N_4$: 299.12967, found: 299.12970.

*5(6)-(E)-(Phenylazo)-2-(4-N,N-dimethylaminophenyl)-1H-benzimidazole (**49**)*

Violet powder; yield: 58%; mp: 242°C to 244°C; IR (ATR): ν_{max} in cm^{-1}: 3469 (NH), 3060 (CH's), 2918, 2850 (CH_3's), 1607 (CN), 1453, 1428 (N═N). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 2.99 (s, 6H, 2(CH_3)), 6.65 (d, J = 6.0 Hz, 2H), 6.87 (q, J = 6.0 Hz, 4H), 7.17-7.23 (m, 2H), 7.40 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 6.0 Hz, 2H) 7.66 (d, J = 9.0 Hz, 1H), 10.98 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 63.4, 104.0, 112.3, 114.1, 117.6, 122.3, 125.5, 127.9, 128.0, 129.7, 135.7, 144.5, 145.3, 151.6, 153.7, 154.2 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{21}H_{20}N_5$: 342.17187, found: 342.17186.

*5(6)-(E)-(Phenylazo)-2-(3,4-dimethoxyphenyl)-1H-benzimidazole (**50**)*

Violet powder; yield: 64%; mp: 232°C to 235°C; IR (ATR): ν_{max} in cm^{-1}: 3447, 3337 (NH), 3228, 3201 (CH's), 2889 (CH_3), 1638 (CN), 1492, 1480 (N═N), 1386 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.62 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 6.95 (d, J = 8.1 Hz, 1H), 7.10-7.34 (m, 6H), 7.51 (d, J = 7.2 Hz, 1H), 7.60 (d, J = 7.2 Hz, 2H), 7.68 (d, J = 7.5 Hz), 12.15 ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 55.8, 56.1, 110.1, 111.9, 112.2, 116.7, 122.3, 122.9, 124.5, 129.5, 131.6, 140.75, 140.78, 145.1 149.5, 150.2, 152.1 152.2 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{21}H_{19}N_4O_2$: 359.14298, found: 359.14284.

*5(6)-(E)-(Phenylazo)-2-(2-aminophenyl)-1H-benzimidazole (**51**)*

Violet powder; yield: 67%; mp: 222°C to 224°C; IR (ATR): ν_{max} in cm^{-1}: 3458, 3328 (NH), 3186 (CH's), 1635 (CN), 1510, 1455 (N═N), 1340 (C═C) ppm. ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.39 (s, 2H, NH_2), 7.50-7.57 (m, 2H), 7.58-7.21 (m, 2H), 7.68 (d, J = 7.2 Hz, 2H), 7.78-7.84 (m, 4H), 7.92 (d, J = 7.2 Hz, 2H), 8.03 (d, J = 8.1 Hz and J = 1.2 Hz, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 108.0, 112.0, 114.1, 114.2, 114.3, 119.1, 122.0, 123.0, 126.3, 128.4, 128.7, 130.1, 142.7, 143.5, 144.3, 154.2, 156.3 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{19}H_{16}N_5$: 314.13275, found: 314.13197.

*5(6)-(E)-(Phenylazo)-2-(3-aminophenyl)-1H-benzimidazole (**52**)*

Violet powder; yield: 61%; mp: 255°C to 257°C; IR (ATR): ν_{max} in cm^{-1}: 3428, 3338 (NH), 2918, 2845 (CH's), 1630 (CN), 1505, 1494 (N═N), 1381 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 4.81 (s, 2H, NH_2), 6.74 (d, J = 7.5 Hz, 1H), 6.94 (s, 1H), 7.07 (q, J = 7.2 Hz, 1H), 7.14 (t, J = 3.3 Hz, 1H), 7.22 (s, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.48-7.62 (m, 6H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 108.0, 114.2, 114.4, 114.6, 115.2, 116.3, 126.4, 126.9, 130.0, 130.5, 131.6, 137.5, 138.2, 144.0, 148.5, 149.5, 150.2 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{19}H_{16}N_5$: 314.13275, found: 314.13205.

*5(6)-(E)-(Phenylazo)-2-(4-aminophenyl)-1H-benzimidazole (**53**)*

Violet powder; yield: 60%; mp: 260°C to 263°C; IR (ATR): ν_{max} in cm^{-1}: 3350, 3221 (NH), 2900 (CH's), 1606 (CN), 1505, 1478 (N═N), 1368 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 4.15 (s, 2H, NH_2), 6.50-6.56 (m, 2H), 6.92-7.19 (m, 4H), 7.83-8.24 (m, 6H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 111.7, 115.3, 115.6, 119.9, 120.5, 122.6, 127.3, 129.6, 130.1, 137.9, 141.8, 144.6, 147.5, 151.9, 152.3, 154.2 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{19}H_{16}N_5$: 314.13275, found: 314.13189.

*5(6)-(E)-(Phenylazo)-(2-nitrophenyl)-1H-benzimidazole (**54**)*

Dark Violet powder; yield: 60%; mp: 260°C to 262°C; IR (ATR): ν_{max} in cm^{-1}: 3457, 3333 (NH), 3176, 3060 (CH's), 1626 (CN), 1567, 1343 (NO_2), 1519, 1497 (N═N), 1457 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 7.10 (t, J = 6.0 Hz, 1H), 7.15–7.29 (m, 5H), 7.37-7.53 (m, H), 12.17 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 112.0, 113.2, 113.8, 119.0, 119.2, 123.8, 126.8, 127.5, 28.7, 128.8, 130.0, 132.0, 134.4, 139.9, 142.0, 145.2, 148.0, 149.0, 151.1 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{19}H_{14}N_5O_2$, 344.10692; found: 344.10612.

*5(6)-(E)-(Phenylazo)-(3-nitrophenyl)-1H-benzimidazole (**55**)*

Dark Violet powder; yield: 67%; mp: 245°C to 248°C; IR (ATR): ν_{max} in cm^{-1}: 3455, 3331, (NH) 3135, 3056, 3093 (CH's) 1626 (CN) 1567, 1443 (NO_2) 1508, 1456 (N═N) 1567 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 6.50-6.60 (m, 4H), 6.92-7.19 (m,

5H), 8.04-8.15 (m, 3H), 10.41 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 101.9, 103.6, 108.3, 108.4, 110.4, 11.0, 129.5, 129.7, 130.2, 139.2, 149.6, 150.1, 159.9, 160.0, 161.2 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{14}N_5O_2$, 344.10692; found: 344.10603.

*5(6)-(E)-(Phenylazo)-(4-nitrophenyl)-1H-benzimidazole (**56**)*

Dark Violet powder; yield: 60%; mp: 242°C to 244°C; IR (ATR): ν_{max} in cm^{-1}: 3465, 3343 (NH), 3140, 3021 (CH's), 1629 (CN), 1565, 1385 (NO_2), 1508, 1457 (N═N), 1565 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 7.13 (d, J = 9 Hz, 1H), 7.48-7.52 (m, 4H), 7.80 (d, J = 9 Hz, 2H), 7.91 (d, J = 3 Hz, 1H), 8.01 (s, 2H), 8.50(s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 120.6, 122.6, 124.6, 127.4, 129.6, 130.1, 131.0, 139.6, 140.2, 140.9, 141.8, 145.6, 147.1, 148.6, 152.3 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{14}N_5O_2$, 344.10692; found: 344.10614.

*5(6)-Aminophenyl-1H-benzimidazole (**57**)*

White powder; yield: 65%; mp: 232°C to 233°C; IR (ATR): ν_{max} in cm^{-1}: 3425 (NH), 2929 (CH's), 1631 (CN), 1523 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 4.33 (s, 1H, NH), 7.78 (d, J = 9.0 Hz, 1H), 6.95(s, 1H, NH), 7.16 (d, J = 6.0 Hz, 1H), 7.22 (d, J = 6.0 Hz, 1H), 7.28 (t, J = 6.0 Hz, 2H), 7.33 (d, J = 6.0 Hz, 2H), 8.0 (s, 1H), 9.42 (sb, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 108.5, 111.9, 116.0, 127.4, 129.4, 129.6, 131.4, 134.3, 137.2, 143.2, 143.8 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{13}H_{12}N_3$: 210.10312, found: 210.10372.

*5(6)-Aminophenyl-2-phenyl-1H-benzimidazole (**58**)*

White powder; yield: 73%; mp: 290°C to 291°C; IR (ATR): νmax in cm^{-1}: 3335 (NH), 3216 (CH's), 1598 (CN), 1516, 1479 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 7.31 (t, J = 7.5 Hz, 1H), 7.41 (q, J = 7.5 and J = 1.2 Hz, 3H), 7.44 (s, 1H), 7.48 (q, J = 7.8 and J = 2.4 Hz, 1H), 7.55 (q, J = 7.2 and J = 2.5 Hz, 2H), 7.65 (q, J = 7.8 and J = 4.5 Hz, 1H), 8.08 (dd, J = 8.7 and J = 1.5 Hz, 1H), 8.26 (dd, J = 8.4 and J = 1.2 Hz, 1H), 8.30 (d, J = 2.4 Hz, 2H), 8.32 (d, J = 1.5 Hz, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 109.2, 115.6, 119.2, 123.58, 127.6, 128.4, 128.6, 129.1, 129.5, 130.2, 131.5, 134.6, 143.2, 145.2, 153.2 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{16}N_3$: 286.13442, found: 286.13434.

*5(6)-Aminophenyl-2-(4-N,N-dimethylaminophenyl)-1H-benzimidazole (**59**)*

Light green powder; yield: 86%; mp: 266°C to 269°C; IR (ATR): ν_{max} in cm^{-1}: 3350, 3215 (NH), 3071, 2887 (CH's, CH_3), 1607 (CN), 1518 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 2.99 (s, 3H), 3.03 (s, 3H), 3.82 (s, 1H, NH), 6.88 (d, J = 8.7 Hz, 2H), 7.05 (t, J = 4.5 Hz, 2H), 7.14 (d, J = 7.5 Hz, 1H), 7.27 (t, J = 7.5 Hz, 2H), 7.37 (d, J = 8.4 Hz, 1H), 7.57 (d, J = 8. 7 Hz, 1H), 7.94 (d, J = 8.4 Hz, 2H), 8.45 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 21.5, 112.5, 117.0, 122.5, 122.7, 123.0, 125.4, 128.4, 129.3, 129.7, 130.2, 132.6, 143.9, 148.1, 152.0, 152.5 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{21}H_{21}N_4$: 3289.17662, found: 329.17549.

*5(6)-Aminophenyl-2-(3,4-dimethoxyphenyl)-1H-benzimidazole (**60**)*

Light green powder; yield: 69%; mp: 203°C to 205°C; IR (ATR): ν_{max} in cm^{-1}: 3369 (NH), 3001, 2933, (CH's, CH_3), 1632, 1598 (CN), 1176, 1023 (C═O), 1496 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 3.82 (s, 3H), 3.86 (s, 3H), 6.75 (t, J = 7.2 Hz, 1H), 6.97 (s, 2H), 7.01 (d, J = 7.8 Hz, 1H), 7.19 (t, J = 8.1 Hz, 2H), 7.39 (d, J = 1.8 Hz, 1H), 7.43 (d, J = 9 Hz, 1H), 7.56 (dd, J = 8.4 and J = 1.8 Hz, 1H), 8.01 (s, 1H), 9.79 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 55.9, 56.3, 109.8, 111.2, 111.7, 116.1, 118.3, 118.3, 119.3, 120.8, 126.6, 129.6, 129.6, 129.9, 132.6, 137.1, 143.7, 156.2, 159.3 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{21}H_{20}N_3O_2$: 346.15555, found: 346.15558.

*5(6)-Aminophenyl-2-(2-aminophenyl)-1H-benzimidazole (**61**)*

Light yellow powder; yield: 72%; mp: 210°C to 212°C; IR (ATR): ν_{max} in cm^{-1}: 3468, 3306 (NH), 3065, 2922, 2852 (CH's), 1660 (CN), 1591 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 5.81 (s, 2H, NH_2), 7.54-7.59 (m, 6H), 7.72-7.94 (m, 3H), 7.97-8.01 (m, 2H), 8.2 (s, 1H), 9.10 (s, 1H, NH), 10.2 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 106.0, 112.1, 114.7, 117.0, 117.6, 120.7, 124.1, 127.6, 128.4, 132.3, 134.9, 135.1, 135.5, 148.3, 149.2, 155.6 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{17}N_4$: 301.17903, found: 301.17987.

*5(6)-Aminophenyl-2-(3-aminophenyl)-1H-benzimidazole (**62**)*

Light yellow poder; yield: 87%; mp: 166°C to 168°C. IR (ATR): ν_{max} in cm^{-1}: 3429 (NH), 2900 (CH's), 1605 (CN), 1518 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.18 (s, 2H, NH_2), 7.01 (d, J = 7.2 Hz, 1H), 7.39 (s, 1H), 7.51-7.58 (m, 4H), 7.95-8.41 (m, 6H), 10.22 (s, 1H, NH), 10.62 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 108.1, 115.6, 116.7, 119.3, 122.117, 122.119, 124.0, 124.3, 129.2 130.2, 130.8., 134.**7**, 135.1 143.2, 147.4, 148.9, 155.6, ppm. HR-DART- MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{17}N_4$: 301.17903, found: 301.17987.

*5(6)-Aminophenyl-2-(4-aminophenyl)-1H-benzimidazole (**63**)*

Light yellow powder; yield: 77%; mp: 178°C to 180°C; IR (ATR): ν_{max} in cm^{-1}: 3335, 3216 (NH), 3105 (CH's), 1598 (CN), 1580 (C═C). ^1H NMR (300 MHz, DMSO-d_6/TMS) δ: 5.06 (s, 2H, NH_2), 7.04 (d, J = 6.0 Hz, 2H), 7.14-7.22 (m, 4H), 7.61-7.67 (m, 4H), 8.02 (d, J = 9.0 Hz, 2H, NH), 10.26 (s, 1H, NH), 12.56 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 110.5, 114.1, 114.5, 119.6, 120.1, 122.0, 127.6, 128.4, 130.0, 131.6, 132.5, 136.1, 144.1 146.1, 158.4 ppm. HR-DART- MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{17}N_4$: 301.17903, found: 301.18937.

5(6)-Aminophenyl-2-(2-nitrophenyl)-1H-benzimidazole (64)

Dark violet powder; yield: 78%; mp: 210–213°C; IR (ATR): ν_{max} in cm^{-1}: 3287, 3253 (NH), 3109, 3084 (CH's), 1593 (CN), 1555 (C═C), 1505, 1336 (NO_2). ^1H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 6.73-7.00 (m, 4H), 7.54-7.90 (m, 8H), 8.50 (s, 1H), 10.40 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 113.3, 115.2, 115.4, 124.6, 125.4, 126.0, 127.6, 128.3, 130.1, 132.8, 136.4, 139.5, 141.2, 148.9, 150.0 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{17}N_4$: 331.11950, found: 331.12026.

5(6)-Aminophenyl-2-(3-nitrophenyl)-1H-benzimidazole (65)

Dark violet powder; yield: 77%; mp: 230°C to 231°C; IR (ATR): ν_{max} in cm^{-1}: 3376, 3200 (NH), 3085, 2926, 2865 (CH's), 1665 (CN), 1598 (C═C), 1526, 1348 (NO_2). ^1H NMR (300 MHz, DMSO-d_6/TMS + CDCl/TMS) δ: 5.61 (s, 1H, NH), 7.02 (d, J = 6.0 Hz, 1H), 7.23-7.31 (m, 4H), 7.48-7.56 (m, 4H), 7.75 (dd, J = 6.0 and 3.0 Hz, 2H), 8.20 (d, J = 6.0 Hz, 1H), 12.94 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 109.9, 113.9, 117.9, 122.1, 123.1, 123.6, 124.1, 127.9, 128.9, 130.1, 131.6, 134.8, 135.1, 135.7, 141.9, 147.5, 147.7 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{15}N_4O_2$: 331.11950, found: 331.12026.

*5(6)-Aminophenyl-2-(4-nitrophenyl)-1H-benzimidazole (**66**)*

Dark violet powder; yield: 72%; mp: 223°C to 225°C; IR (ATR): ν_{max} in cm^{-1}: 3382 (NH), 3100, 3067, 2922, 2852 (CH's), 1704 (CN), 1598 (C═C), 1518, 1344 (NO_2). ^1H NMR (300 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 4.81 (s, 1H, NH), 6.61 (s, 1H), 6.69 (d, J = 9 Hz, 1H), 6.70 (d, J = 9 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 8.27-8.37 (m, 6H), 8.41 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS + $CDCl_3$/TMS) δ: 113.1, 115.2, 115.5, 124.69, 125.2, 125.6, 125.9, 130.2, 130.7, 132.2, 135.5, 139.5, 141.0, 149.9, 155.1 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{19}H_{15}N_4O_2$: 331.12026, found: 331.13750.

*5(6)-Ethanolamine-1H-benzimidazole (**67**)*

Violet powder; yield: 67%; mp: 320°C to 321°C; IR (ATR): ν_{max} in cm^{-1}: 3266 (NH), 3168, 2897 (CH's, CH_2), 1665 (CN), 1561 (C═C), 1100 (OH), 1000 (C—O). ^1H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.23 (q, J = 5.4 Hz, 2H), 3.42 (q, J = 5.4 Hz, 2H), 3.56 (d, 1H, OH), 3.73 (s, 1H, NH), 4.69 (s, 1H, NH), 7.38 (d, J = 9.0 Hz, 1H), 7.81 (d, J = 9 Hz, 1H), 8.60 (d, J = 1.8 Hz, 1H), 8.88 (d, J = 1.8 Hz, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 46.0, 60.1, 109.6, 113.1, 118.3, 129.0, 136.8, 139.8, 143.2 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_9H_{11}N_3O$: 177.09021, found: 177.08951.

*5(6)-Ethanolamine-2-phenyl-1H-benzimidazole (**68**)*

White powder; yield: 68%; mp: 210°C to 212°C; IR (ATR): ν_{max} in cm^{-1}: 3244 (NH), 3107, 3051, 2922 (CH's, CH_2), 1663 (CN), 1513 (C═C), 1181 (C—O), 1107 (OH). ^1H NMR (300 MHz, DMSO-$_{d6}$/TMS) δ: 3.255 (q, J = 5.4 Hz, 2H), 3.60 (q, J = 5.4 Hz, 2H), 5.43 (s, 1H, OH), 6.81 (d, J = 6.9 Hz, 2H), 7.24 (d, J = 1.5 Hz, 1H), 7.89-8.02 (m, 1H), 8.03-8.05 (m, 3H), 8.15 (d, J = 6.9 Hz, 1H), 8.26 (d, J = 6.9 Hz, 2H), 9.05 (s, 1H, NH), 10.94 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 45.5, 59.5, 113.3, 124.2, 124.4, 124.6, 127.2, 128.9, 130.4, 131.0, 140.4, 149.6, 151.0 ppm. HR-DART-MS *m/z* ($[M + 1]^+$): Calcd. for $C_{15}H_{16}N_3O$: 254.12934, found: 254.12820.

*5(6)-(Ethanolamino)-2-(4-N,N-dimethylaminophenyl)-1H-benzimidazole (**69**)*

Green light powder; yield: 73%; mp: 268°C to 269°C; IR (ATR): ν_{max} in cm^{-1}: 3331 (NH), 3059, 2857 (CH's, CH_3), 1640, 1608 (CN), 1506 (C═C), 1174, 1110 (C—O), 1062 (OH). ^1H NMR (300 MHz, DMSO-d_6/TMS) δ: 2.80 (s, 1H, OH), 3.12 (t, J = 5.7 Hz, 2H), 3.61 (t, J = 5.7 Hz, 2H), 5.87 (s, 2H, NH), 6.65 (s, 1H), 6.67 (s, 1H), 6.80 (d, J = 8.7 Hz, 2H), 7.33 (d, J = 9 Hz, 1H), 7.91 (d, J = 8.7 Hz, 2H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 42.2, 46.3, 59.5, 93.2, 111.8, 113.9, 114.9, 127.5, 128.7, 136.3, 146.2, 148.7, 151.5 ppm. HR-DART-MS m/z ($[M + 1]^+$): Calcd. for $C_{17}H_{21}N_4O$: 297.17154, found: 297.17161.

*5(6)-(Ethanolamino)-2-(3,4-dimethoxyphenyl)-1H-benzimidazole (**70**)*

Dark green powder; yield: 78%; mp: 259°C to 261°C; IR (ATR): νmax in cm^{-1}: 3375 (NH), 2933, 2839 (CH's, CH_3), 1636 (CN), 1510 (C═C), 1177, 1153 (C—O), 1105

(OH). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.11 (t, J = 6 Hz, 2H), 3.22 (s, 1H, OH), 3.60 (t, J = 6 Hz, 2H), 3.81 (s, 3H), 3.85 (s, 3H), 5.68 (s, 1H, NH), 6.57 (d, J = 8.7 and J = 2.1 Hz, 1H), 6.60 (s, 1H), 7.07 (d, 1H, J = 8.7 Hz), 7.28 (d, J = 8.4 Hz, 1H), 7.61 (dd, J = 8.1 and J = 8.1 Hz, 1H), 7.67 (d, J = 1.8 Hz, 1H), 9.89 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 46.7, 55.9, 56.1, 60.0, 104.1, 109.9, 110.7, 112.2, 112.4, 119.9, 122.4, 128.3, 140.2, 142.9, 149.2, 149.4, 151.5 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{17}H_{20}N_3O_3$: 314.14264, found: 314.14178.

*5(6)-(Ethanolamino)-2-(2-aminophenyl)-1H-benzimidazole (**71**)*

Green-yellow light powder; yield: 57%; mp: 240°C to 242°C; IR (ATR): ν_{max} in cm^{-1}: 3479, 3366 (NH), 3000, 2920 (CH's, CH_2), 1634 (CN), 1606 (C═C), 1160, 1100 (CO), 1092 (OH). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ:2.8 (s, 1HH, OH), 3.23 (q, J = 6.9 Hz, 2H), 3.43 (q, J = 6.9 Hz, 2H), 6.76 (d, J = 3.0 Hz, 1H), 6.87-6.95 (m, 2H), 7.29-7.31 (m, 1H), 7.34 (q, J = 9.3 and J = 2.4 Hz, 1H), 7.72 (d, J = 9.0 Hz, 1H), 8.45 (s, 1H, NH), 8.61 (s, 2H, NH_2), 9.8 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 40.8, 60.4, 91.1, 101.3, 112.8, 113.7, 116.8, 121.5, 124.7, 130.3, 134.6, 134.7, 144.6, 153.5, 154.2 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{15}H_{17}N_4O$: 269.14024, found: 269. 14076.

*5(6)-(Ethanolamino)-2-(3-aminophenyl)-1H-benzimidazole (**72**)*

Green-yellow light powder; yield: 67%; mp: 234°C to 236°C; IR (ATR): ν_{max} in cm^{-1}: 3252 (NH), 3108, 2925, (CH's, CH_2), 1664 (CN), 1516 (C═C), 1181 (C—O), 1109 (OH). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.24 (t, J = 5.7 Hz, 2H, OH), 3.63 (t, J = 5.7 Hz, 2H), 5.05 (s, 2H, NH_2), 6.03 (s, 1H, OH), 6.50 (d, J = 2.4 Hz, 1H), 6.52-6.80 (q, J = 8.4 and J = 2.1 Hz, 1H), 7.22 (s, 1H), 7.26 (s, 1H), 7.64-7.79 (m, 2H), 7.96 (q, J = 7.8 and J = 1.5 Hz, 1H), 9.04 (s, 1H, NH), 13.14 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 47.9, 59.0, 106.5, 116.1, 116.5, 117.4, 119.6, 124.2, 124.7, 131.4, 131.9, 133.2, 134.9, 135.2, 147.9, 157.0 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{15}H_{17}N_4O$: 269.14024, found: 269.14076.

*5(6)-(Ethanolamino)-2-(4-aminophenyl)-1H-benzimidazole (**73**)*

Green-yellow light powder; yield: 61%; mp: 250°C to 252°C; IR (ATR): ν_{max} in cm^{-1}: 3353 (NH), 3106, 2939, (CH's, CH_2), 1621 (CN), 1596 (C═C). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.12 (q, J = 5.7 Hz, 2H), 3.27 (s, 1H, OH), 3.56 (d, J = 4.5 Hz, 2H), 3.84 (s, 1H, NH), 4.82 (s, 2H, NH_2), 6.62 (d, J = 8.4 Hz, 1H), 7.69 (q, J = 9.6 and J = 2.7 Hz, 1H), 8.13 (s, 2H), 8.19 (d, J = 3.2 Hz, 1H), 8.37 (s, 2H), 9.56 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 45.5, 55.5, 101.4, 113.8, 113.9, 114.9, 116.1, 128.4, 133.1, 137.1, 141.9, 145.8, 153.7 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{15}H_{17}N_4O$: 269.14024, found: 269.14076.

*5(6)-(Ethanolamino)-2-(2-nitrophenyl)-1H-benzimidazole (**74**)*

Dark violet powder; yield: 62%; mp: 230°C to 233°C; IR (ATR): ν_{max} in cm^{-1}: 3347 (NH), 3105, 2922 (CH's, CH_2), 1701 (CN), 1595 (C═C), 1520, 1344 (NO_2), 1159, 1105 (C—O), 1067 (OH). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 3.13 (q, J = 6.8 and 1.3 Hz, 2H), 3.55 (q, J = 6.8 and 1.3 Hz, 2H), 3.91 (s, 1H, OH), 4.92 (sb, 2H, NH), 6.65 (d, J = 9.0 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.75-7.88 (m, 3H), 8.22 (d, J = 7.8 Hz, 1H), 8.48 (d, J = 7.2 Hz, 1H), 8.88 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 45.9, 62.0, 104.0, 115.2, 115.5, 124.9, 125.2, 125.6, 130.2, 130.7, 132.2, 135.5, 139.5, 141.0, 149.9, 155.1 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{15}H_{15}N_4O_3$: 299.11441, found: 299.11361.

*5(6)-(Ethanolamino)-2-(3-nitrophenyl)-1H-benzimidazole (**75**)*

Dark violet powder; yield: 78%; mp: 226°C to 229°C; IR (ATR): ν_{max} in cm^{-1}: 3358, 3226 (NH), 3085, 2930, 2868 (CH's, 5CH), 1720 (CN),1635 (C═C), 1525, 1348 (NO), 1196, 1099 (C—O), 1061 (OH) ppm. ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 2.60 (s, 1H, OH), 3.14 (d, J = 5.7 Hz, 2H), 3.62 (d, J = 5.7 Hz, 2H), 4.72 (s, 2H, NH), 6.63-6.67 (m, 2H), 7.38 (d, J = 8.4 Hz, 1H), 7.77 (t, J = 7.8 Hz, 1H), 8.22 (d, J = 7.8 Hz, 1H), 8.48 (d, J = 7.8 Hz, 1H), 8.89 (s, 1H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 44.1, 55.5, 102.9, 113.9, 116.1, 122.4, 124.6, 130.1, 130.5, 130.6, 130.9, 137.1, 140.8, 148.7, 153.7 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{15}H_{15}N_4O_3$: 299.11441, found: 299.11423.

*5(6)-(Ethanolamino)-2-(4-nitrophenyl)-1H-benzimidazole (**76**)*

Dark violet powder; yield: 78%; mp: 241°C to 245°C; IR (ATR): ν_{max} in cm^{-1}: 3269, 3231 (NHas), 3188 (NHsim), 2933, 2877 (CH's, CH_2), 1724 (CN), 1634 (C═C), 1514, 1340 (NO_2), 1192, 1135 (C—O), 1108 (OH). ^{1}H NMR (300 MHz, DMSO-d_6/TMS) δ: 2.83 (s, 1H, OH), 3.28 (q, J = 6.6 and 3.2 Hz, 2H), 3.75 (d, J = 6.6 Hz, 2H), 4.68 (s, 1H, NH), 5.23 (s, 1H, NH), 7.12 (d, J = 8.1 Hz, 1H), 7.24 (s, 1H), 7.41 (s, 1H), 7.53–7.69 (m, 4H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6/TMS) δ: 45.9, 59.2, 100.3, 107.1, 108.6, 115.2, 127.7, 131.3, 136.9, 139.0, 145.8, 150.0, 153.2 ppm. HR-DART-MS *m/z* ([M + 1]$^+$): Calcd. for $C_{15}H_{15}N_4O_3$: 299.11441, found: 299.11472.

5 | BIOLOGICAL ASSAYS

5.1 | Materials and general methods

5.1.1 | Preparation of drugs and test compounds

The synthesized compounds and reference drugs were dissolved in 1 mL of DMSO and diluted to 500 μg/100 mL with a culture medium.

5.1.2 | Microorganisms

S aureus ATCC 6538 and ATCC 43300, *E coli* ATCC 8739, and *C albicans* ATCC 10231 strains were used. The qualitative antimicrobial activity technique was carried out according to the literature method.[37]

5.1.3 | MIC assay by microdilution and viable count

The MIC values were determined by the microdilution test using a methicillin-resistant MRSA strain (ATCC 43300). The biological assay was carried out regarding a reported method[37] with slight modification.

5.1.4 | Preparation of cultures and inoculum

Two days before the experiment, the test strains were cultivated on sheep blood agar plate and incubated for 20 to 24 hours at 35°C to 37°C. All bacterial strains were treated in the same manner from this point on. From the resulting growth on each plate, selected and isolated colonies were spread, using a sterile disposable loop, onto a fresh Mueller Hinton agar plate and incubate for 20 to 24 hours at 35°C to 37°C.

5.1.5 | Preparation of assay plates with medium and drugs

One hundred microliter of Mueller Hinton broth was added to each well of 96-well microtiter plate at room temperature. Then, using a multichannel pipet, 100 μL of the appropriate compound stock solution was added to the first well of each row and serially diluted each row twofold across the plate to the 11th well, discarding the remaining 100 μL to obtain compound concentrations ranging from 131.5 μg in the first well to 0.12 μg in the 11th well. Well, the 12th is the growth control and contains no drug.

5.1.6 | Preparation of cultures and assay plates

Using a sterile inoculating loop several colonies wire transfer from an overnight plate culture into 5 mL of SSS. Inoculum density was adjusted to tube 0.5 of McFarland nephelometer (1-2 × 10^8 UFC mL^{-1}). All test and control strains were handled in the same manner. The above suspension was diluted by adding 200 μL into 28 mL of Mueller Hinton broth. Afterward, 100 μL of the resulting inoculum suspension was added to all 96-wells plates. Additionally, 100 μL of DMSO solution prepared by dissolving 100 μL of DMSO into 1.900 mL of Mueller Hinton broth, as no-drug containing well control was added to the 12th well. These plates were incubated in an ambient air incubator for 24 hours at 35°C to 37°C (85% relative humidity). After, the minimum inhibitory concentration-MIC values were read and recorded.

5.1.7 | Determination of microbial reduction percentage

After the MIC value was recorded; the 96-well MIC plates were shaking and re-incubated for an additional 4 hours at 35°C to 37°C. Then, a 100-μL sample from each well with no visible growth in the MIC assay was removed and spread on a sheep blood agar plate (in duplicate). Afterward, the inverted agar plates were incubating for 24 hours at 35°C to 37°C. Elapsed this time, colonies were counted on the agar plate and reported as the % reduction (% R) using the following equation:

$$\%R = \frac{UFC1 - UFC2}{UFC1} \times 100. \quad (1)$$

where UFC1 = UFC of 100 μL of no-drug control well and.

UFC2 = UFC of 100 μL of drug-containing well.

5.1.8 | Determination of viable count

A 1:100 dilution of the 0.5 McFarland solution (0.1 mL + 9.9 mL of standard saline solution SSS) was made. Once the concentration was adjusted to 10^6, 10^5, 10^4 UFC, and 100 μL of each solution was sown onto the agar plates, and after their incubation for 24 hours at 35°C, the number of colonies were counted and recorded.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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