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Microwave-assisted synthesis of N-substituted cyclic imides and their evaluation for anticancer and anti-inflammatory activities

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ABSTRACT

A number of N-substituted cyclic imides **3a–e**, **5a–e**, **7a–d**, and **9a–e** have been synthesized in very high yields, by condensation of various diacids **2**, **4**, **6**, and **8** with different amines under microwave irradiation. These compounds were screened for anticancer and anti-inflammatory activities, and compounds **3c**, **3e**, **5c**, **9c**, and **9d** exhibited anticancer activity against colon (COLO 205) cancer better than 5-fluorouracil and mitomycin-C, and compound **9b** exhibited anti-inflammatory activity better than standard drug phenyl butazone.

Imide moiety is an integral part of structures of various important molecules such as fumaramidmycin,[1,2] granulatimide,[3] isogranulatimide,[4] rebeccamycin,[5] and thalidomide.[6,7] These molecules are reported to exhibit wide variety of biological activities such as antitumor,[8,9] anti-inflammatory,[10] and antimicrobial.[11] Apart from biological activities, imide derivatives are useful in the reactions[12] involving condensation, alkylation, acylation, and cyclocondensation. In search of biologically active molecules microwave technology is playing an important role, as time required for synthesizing new molecules by using microwave technology is much less as compared to conventional methods, and hence large number of compounds can be synthesized in a short period of time for biological evaluation.[13]

In continuation of our efforts in search of potent molecules exhibiting anticancer or anti-inflammatory activity, we have synthesized a number of bicyclo heterocyclic imides and screened them for anticancer and anti-inflammatory activity which we wish to report in this paper.

A number of bicyclo heterocyclic imides, that is, **3a–e**, **5a–e**, **7a–d**, and **9a–e** have been synthesized by following reaction (Scheme 1) and using the reaction procedure reported earlier by our group.[14] Condensation of diacids **2**, **4**, **6**, and **8** with amine **1** (Scheme 1) was carried out by mixing diacid with amine in 1:1 molar ratio, and then irradiating them in microwave oven for 2–5 min at a power level of 600 W. Condensed product so obtained was purified by crystallization from methanol: ethyl acetate (9:1) to give pure product. Irradiation time and percentage yield for **3a–e**, **5a–e**, **7a–d**, and **9a–e** are summarized in Table 1. Physical constants and spectral data of **3d**,**e**, **5d**,**e**,**7d**, and **9d**,**e** are reported in our earlier communication,[14] and for **3a–c**, **5a–c**, **7a–c**, and **9a–c** are reported in this paper.[15] Lipophilicity (*c*Log*P*) values for compounds **3a–e**, **5a–e**, **7a–d**, and **9a–e** were estimated by using ChemDraw Ultra and are reported in Table 1.

Bicycloimides **3a–e**, **5a–e, 7a–d**, and **9a–e** after characterization by spectroscopic means were screened for anticancer activity against four human cancer cell lines,[16,17] that is, liver (HEP-2), colon (SW-620, COLO-205), and CNS (SK-NS-H). Percentage growth inhibition of various cancer cell lines was determined at a concentration of 1×10^{-5} M solution of various compounds and results are summarized in Table 2.

Compounds **3c**, **3e**, **5c**, **9c**, and **9d** exhibited 53%, 45%, 46%, 52%, and 45% growth inhibition of colon (COLO-205) cancer cell line, respectively, as compared to 5-fluorouracil and mitomycin-C, which exhibited 44% and 29% growth inhibition, respectively.

Anti-inflammatory[18] activity evaluation of **3a–e**, **5a–e**, **7a–d**, and **9a–e** was carried out using carrageenan-induced paw oedema model and results are summarized in Table 3. Compound **9b** exhibited 67% (100 mg/kg p.o.), 40% (50 mg/kg p.o.), and 19% (25 mg/kg p.o.), whereas standard drug phenyl butazone exhibited 63% (100 mg/kg p.o.), 37% (50 mg/kg p.o.), and 17% (25 mg/kg p.o.) anti-inflammatory activity, respectively. Anti inflammatory activity of **9b** is better than phenyl butazone.

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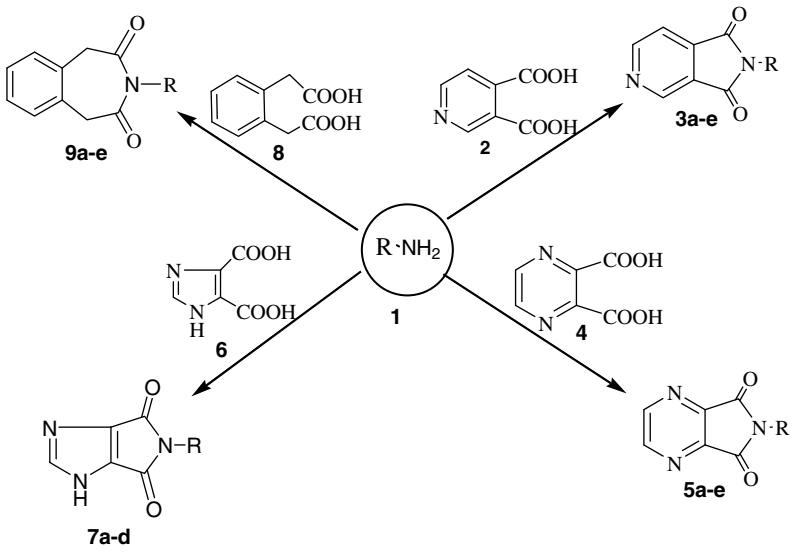
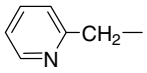
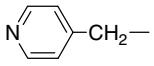
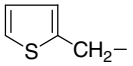
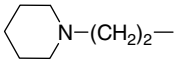
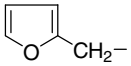


Where R is various substituents;

3a, **5a**, **7a**, **9a**: R=  ; **3b**, **5b**, **7b**, **9b**: R= 

3c, **5c**, **7c**, **9c**: R=  ; **3d**, **5d**, **7d**, **9d**: R= 

3e, **5e**, **9e**: R= 

Scheme 1.

Compound **9b** exhibited good anti-inflammatory activity and compounds **3c**, **3e**, **5c**, **9c**, and **9d** exhibited good anticancer activity against colon (COLO-205) cancer cell line. This may be due to the fact that these molecules meet lipophilicity, stereochemical, and electronic requirements of the target in a better way as compared to other molecules.

A number of bicycloimide derivatives have been synthesized in high yields using microwave irradiation technique and screened for anticancer and anti-inflammatory activity. Compounds **3c**, **3e**, **5c**, **9c**, and **9d** exhibited good anticancer activity against colon (COLO-205) cancer cell line, and compound **9b** exhibited good anti-inflammatory activity.

This report demonstrate our efforts in search of potent molecules which can be synthesized easily. In future, we plan to synthesize bicyclic and tricyclic heterocyclic molecules using simple methods, and then evaluate them for anti-inflammatory and anticancer activities.

In vitro cytotoxicity against human cancer cell lines[16,17]: The human cancer cell lines procured from National Cancer Institute, Frederick, U.S.A. were used in present study. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2 mM glutamine, pH 7.4 supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 U/ml penicillin) in a carbon dioxide incubator (37° C, 5% CO_2, 90% RH). The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% in PBS (pH 7.4) containing 0.02% EDTA). Cells with viability of more than 98%, as determined by trypan blue exclusion, were used for determination of cytotoxicity. The cell suspension of 1×10^5 cells/ml was prepared in complete growth medium.

Stock solution of 4×10^{-2} M of compounds was prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 μg/ml of gentamycin to obtained working test solution of required concentrations. In vitro cytotoxicity against various human cancer cell lines was determined (Monks et. al., 1991)[16] using 96-well tissue culture plates. The 100 μl of cell suspension was added to each well of the 96-well tissue culture plates. The cells were allowed to grow in CO_2 incubator (37° C, 5% CO_2, 90% RH) for 24 h. The test materials in complete growth medium (100 μl) were added after 24 h incubation to the wells containing cell suspension. The plates were further incubated for 48 h (37° C in an atmosphere of 5% CO_2 and 90% relative humidity) in a carbon dioxide incubator after addition of test material, and then the cell growth was stopped by gently layering trichloroacetic acid (50% TCA, 50 μl) on top of the medium in all the wells. The plates were incubated at 4° C for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipetted out and discarded. The plates were washed five times with distilled water to remove TCA, growth medium low molecular weight metabolites, and serum proteins and air-dried. Cell growth was measured by staining with sulforhodamine B dye (Skehan et al.).[17] The adsorbed dye was dissolved in Tris–HCl Buffer (100 μl, 0.01 M, pH 10.4), and plates were gently stirred for 10 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm.

Anti-inflammatory activity[18]: Paw oedema inhibition test was used on albino rats of Charles Foster by adopting the method of

Table 1
Reaction conditions, percentage yield, and lipophilicity values of **3a–e**, **5a–e**, **7a–d**, and **9a–e**

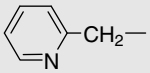
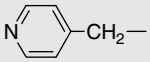
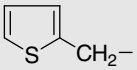
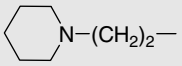
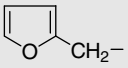
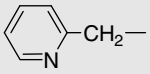
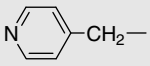
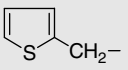
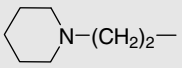
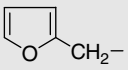
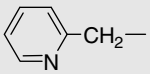
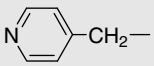
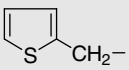
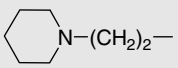
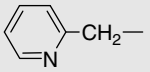
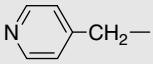
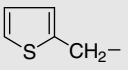
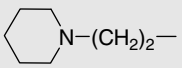
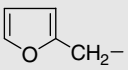
Compound	R	Irradiation time (min)	Yield (%)	Lipophilicity *c*Log *P*
3[a]		4	92	0.684
3b		3	90	0.684
3c		4	94	1.827
3d		4	88	1.587
3e		5	94	1.357
5°		2	96	−0.271
5b		2	98	−0.271
5c		3	96	0.871
5d		5	88	0.632
5e		5	88	0.402
7a		5	96	0.046
7b		5	98	0.046
7c		5	98	1.189
7d		4	94	0.949
9a		2	98	1.419
9b		2	96	1.419
9c		3	92	2.562
9d		3	84	2.276
9e		3	97	2.092

Table 2
Anticancer activity of compounds **3a–e**, **5a–d**, **7a–d**, and 9**a–e**

Compound tested	% Growth inhibition at a conc. 1×10^{-5} M			
	Liver HEP-2	Colon		CNS SK-NS-H
		COLO-205	SW-620	
3a	24	4	33	10
3b	12	12	20	9
3c	24	4	53	12
3d	13	1	0	13
3e	8	0	45	0
5a	16	6	33	11
5b	11	1	36	0
5c	16	7	46	12
5d	12	2	9	10
5e	11	7	12	15
7a	17	6	33	11
7b	26	3	43	1
7c	15	3	23	2
7d	18	10	17	22
9a	11	0	37	0
9b	6	0	22	0
9c	19	4	52	1
9d	15	0	45	0
9e	17	10	17	11
5-FU	40	25	44	56
Mito-C	60	41	29	85

Table 3
Anti-inflammatory activity of compounds **3a–e**, **5a–e**, **7a–d**, and **9a–e**

Compound tested	Dose (mg/kg po)	Anti-inflammatory activity (%)
3[c]	50	27
3b	50	33
3c	50	35
3d	50	12
3e	50	24
5[a]	50	29
5b	50	32
5c	50	31
5d	50	16
5e	50	0
7[a]	50	24
7b	50	21
7c	50	32
7d	50	0
9[a]	50	32
9b	50	40
9b	25	19
9b	100	67
9c	50	34
9d	50	21
9e	50	22
PB	50	37
PB	25	17
PB	100	63

PB denotes phenyl butazone.

Winter et al.[18] Groups of five animals of both sexes (body weight 120–160 g), excluding pregnant females, were given a dose of test compound. Thirty minutes later, 0.20 ml of 1% freshly prepared carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the planter aponeurosis of the hind paw, and the volume was measured by a water plethysmometer apparatus and then measured again 1–3 h later. The mean increase of paw volume at each interval was compared with that of control group (five rats treated with carrageenan but not with test compound) at the same intervals, and percent inhibition value was calculated by the formula given below.

$$\%\text{anti} - \text{inflammatoryactivity} = [1 - D_t/D_c] \times 100$$

D_t and D_c are paw volumes of oedema in tested and control groups, respectively.

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15. IR, ^{1}HNMR and GC-MS data of compounds **3d**, **3e**, **5d**, **5e, 7d**, **9d**, and **9e** are reported in Ref. 14. For other compounds data are reported herewith. (**3a**) mp 152 °C; IR(KBr) cm^{-1} 1713 (–CO–N–CO–), 1595 and 1482 (Ar); ^{1}HNMR (500 MHz DMSO-d_6) 4.15 (s, 2H, CH_2), 7.37–7.42 (m, 2H, py), 7.81–7.84 (m, 1H, py), 7.86–7.87 (d, 1H, py), 8.56–8.57 (d, 1H, py), 8.73–8.74 (d, 1H, py), 9.13 (s, 1H, py); GCMS *m*/*z* 239 (M$^+$ 100%): (**3b**) mp 190 °C; IR(KBr) cm^{-1}1697 (–CO–N–CO–), 1564 and 1489 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 4.19 (s, 2H, CH_2), 7.41–7.42 (d, 2H, py), 7.93–7.94 (d, 1H, py), 7.56–7.57 (d, 2H, py), 8.72–8.73 (d, 1H, py), 9.16 (s, 1H, py); GCMS *m*/*z* 239 (M$^+$ 100%); (**3c**) mp 165 °C; IR(KBr) cm^{-1} 1711 (–CO–N–CO–), 1563 and 1483 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 4.22 (s, 2H, CH_2), 7.05–7.07 (t, 1H, thiop.), 7.20 (s, 1H, thiop.), 7.55–7.56 (d, 1H, py), 7.96–7.97(d, 1H, thiop), 8.73–8.74 (d, 1H, py), 9.24 (s, 1H, py); GCMS *m*/*z* 244 (M$^{+\cdot}$ 100%); (**5a**) mp 148 °C; IR(KBr) cm^{-1}1638 (–CO–N–CO–), 1588 and 1485 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 4.17 (s, 2H, CH_2), 7.37–7.39 (q, 1H, py), 7.44–7.46 (d, 1H, py), 7.81–7.85 (m, 1H, py), 8.57–8.58 (d, 1H, py), 8.66–8.67 (d, 2H, pyrazine); GCMS *m*/*z* 240 (M$^{+\cdot}$ 60%): (**5b**) mp 173 °C; IR(KBr) cm^{-1}1638 (–CO–N–CO–), 1588 and 1447 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 4.17 (s, 2H, CH_2), 7.45–7.46 (q, 2H, py), 8.62–8.63 (q, 2H, py), 8.76 (s, 2H, pyrazine): GCMS *m*/*z* 240 (M$^{+\cdot}$ 59.4%): (**5c**) mp 154 °C; IR(KBr) cm^{-1} 1715 (–CO–N–CO–), 1593 and 1424 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 3.93 (s, 2H, CH_2), 6.82–6.84 (q, 1H, thiop.), 6.95–6.96 (t, 1H, thiop.), 7.31–7.32 (dd, 1H, thiop.), 8.51 (s, 2H, pyrazine): GCMS *m*/*z* 245 (M$^{+\cdot}$ 100%): (**7a**) mp 195 °C; IR (KBr) cm^{-1} 3434 (NH),1699 (–CO–N–CO–), 1625 (C=N), 1560 and 1500 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 4.16 (s, 2H, CH_2), 7.38–7.44 (m, 2H, py), 7.72–7.74 (d, 1H, py), 7.82–7.85 (t, 1H, py), 8.58 (d, 1H, imidaz.): GCMS *m*/*z* 228 (M$^+$ 100%): (**7b**) mp 220 °C; IR(KBr) cm^{-1} 1700 (–CO–N–CO–), 1613 (C=N), 1514 and 1427 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 4.13 (s, 2H, CH_2), 7.45 (s, 2H, py), 7.92 (s, 1H, N=CH–NH), 8.41 (s, 1H, NH), 8.61(s, 2H, py); GCMS *m*/*z* 228 (M$^{+\cdot}$ 10%): (**7c**) mp 225 °C; IR(KBr) cm^{-1} 1703 (–CO–N–CO–), 1646 (C=N), 1544 and 1498 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 4.27 (s, 2H, CH_2), 7.07–7.09 (q, 1H, thiop.), 7.22–6.23 (d, 1H, thiop.), 7.58–7.62 (t, 1H+1H, thiop. + imidz.), 8.53 (bs, 1H, NH): GCMS *m*/*z* 233 (M$^{+\cdot}$ 17%): (**9a**) mp 107 °C; IR (KBr) cm^{-1}1706 (–CO–N–CO–), 1590 and 1440 (Ar); ^{1}HNMR (500 MHz DMSO-d_6) 3.52 (s, 4H, 2× CH_2), 4.08 (s, 2H, CH_2), 7.14–7.19 (m, 4H, Ar), 7.36–7.47 (t, 2H, py), 7.82–7.85 (t, 1H, py), 8.58 (s, 1H, py): GCMS *m*/*z* 266 (M$^{+\cdot}$ 82%): (**9b**) m.p. 175 °C; IR(KBr) cm^{-1}1728 (–CO–N–CO–), 1564 and 1421 (Ar); ^{1}H NMR (500 MHz DMSO-d_6) 3.54 (s, 4H, 2× CH_2), 3.90 (s, 2H, CH_2), 7.15–7.20 (m, 4H, Ar), 7.39–7.40 (d, 2H, py), 8.53-8.54 (q, 2H, py): GCMS *m*/*z* 266 (M$^{+\cdot}$ 20%): (**9c**) mp 185 °C; IR(KBr) cm^{-1} 1687 (–CO–N–CO–), 1515 aa 1448 (Ar); ^{1}HNMR (500 MHz DMSO-d_6) 3.50 (s, 4H, 2× CH_2), 4.12 (s, 2H, CH_2), 7.02–7.04 (q, 1H, thiop.), 7.13–7.17

(m, 1H+4H, thiop. + Ar), 7.49–7.50 (q, 1H, thiop.): GCMS *m*/*z* 271 ($M^{+\cdot}$ 10%).

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