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Original article

# Synthesis and biological activities of some new fluorinated coumarins and 1-aza coumarins

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**Abstract**

A series of new fluorinated coumarins and 1-aza coumarins have been synthesized and the presence of fluorine in these molecules and its effect on their anti-microbial, anti-inflammatory and analgesic activities are discussed. The results of bioassay showed that these newly synthesized compounds containing fluorine exhibit moderate analgesic and excellent anti-inflammatory and potential anti-bacterial and anti-fungal activities, compared to the other halogenated compounds. All the newly synthesized compounds were characterized by elemental analysis, IR, $^{1}$H NMR, $^{13}$C NMR, $^{19}$F NMR, EI-MS, and FAB-MS. The ORTEP diagram of one of the compounds is reported herein.


*Keywords:* Fluorinated coumarin; Fluorinated 1-aza coumarin; Anti-bacterial; Anti-fungal; Analgesic; Anti-inflammatory

## 1. Introduction

Coumarin and its nitrogen analogue 1-aza coumarin (also known as carbostyril) are a class of lactones and lactams, respectively, which are indispensable heterocyclic units to both the chemists and the biochemists. A comparative review of these two systems has been reported recently, for their natural occurrence, anti-microbial, anti-inflammatory, anti-cancer and other miscellaneous properties [1]. Many coumarin derivatives are known for their special property of scavenging Reactive Oxygen Species (ROS) and have been found to be inhibitors of cyclooxygenase and lipoxygenase in the arachidonic acid pathway of inflammation suppression [2]. Non steroidal anti-inflammatory drugs (NSAIDs) have a broad spectrum of effects in acute pain management and target the cyclooxygenase enzyme. Several coumarin derivatives have been reported for their significant anti-inflammatory activities and their ability to inhibit these enzymes in inhibiting inflammation [3–5]. The potential of coumarin derivatives as anti-inflammatory agents has been explored in our laboratory, by incorporating biocompatible pharmacophores like vanillyl, cyanoester and paracetamol, at the allylic position with respect to C3–C4 double bond of the coumarin moiety [6,7].

1-Aza coumarin derivatives, which ultimately metabolize as the corresponding 8-hydroxy coumarins in the biological system are therefore found to be very good anti-inflammatory and analgesic agents [8]. The triheterocyclic thiazoles synthesized from 4-aminomethyl carbostyrils and 3-bromoacetyl coumarins [9] in our laboratory were found to exhibit promising anti-inflammatory and analgesic activities even after 24 h.

Interest in coumarins and 1-aza coumarins as antibiotics is due to the recent observations that these are potent inhibitors of bacterial DNA gyrase, which is involved in cell growth [10,11]. Many coumarin and 1-aza coumarin derivatives, with variety of substituents at 4-position, with very good anti-bacterial activity have been reported from our laboratory [12–15].

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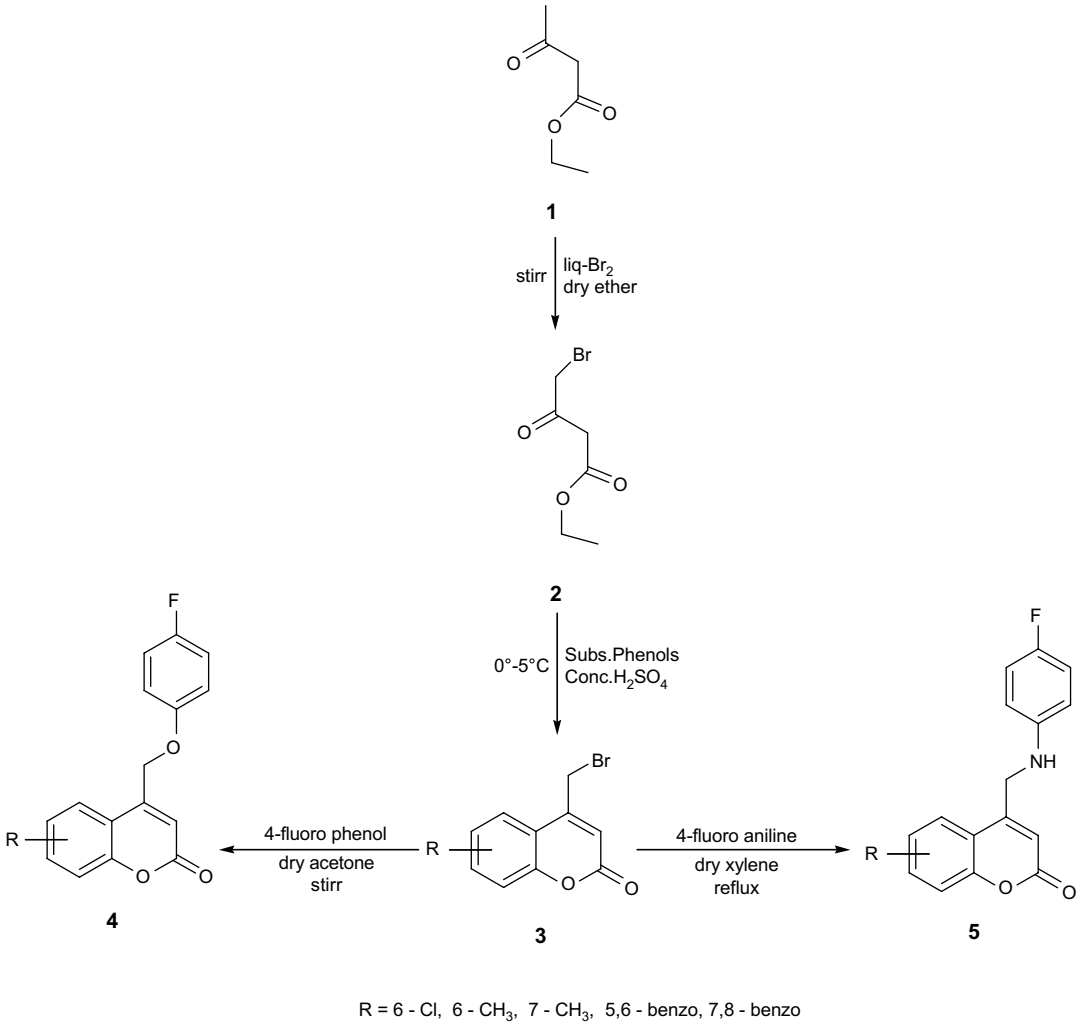
Incorporation of fluorine into organic molecules can lead to distinctive modification in their biological properties [16]. Fluorinated heterocycles have been found to exhibit enhanced enzyme inhibiting [17] and anti-microbial activities, as compared to their non-fluorinated analogues [18,19]. A series of 3-amino carbostyril derivatives containing fluorine have been reported as promising potassium (Maxi-K) channel openers, targeted for neuroprotection [20].

Hence the purpose of the present work was to explore and develop the new templates containing fluorine, coumarin and 1-aza coumarin, with three points of molecular diversity.

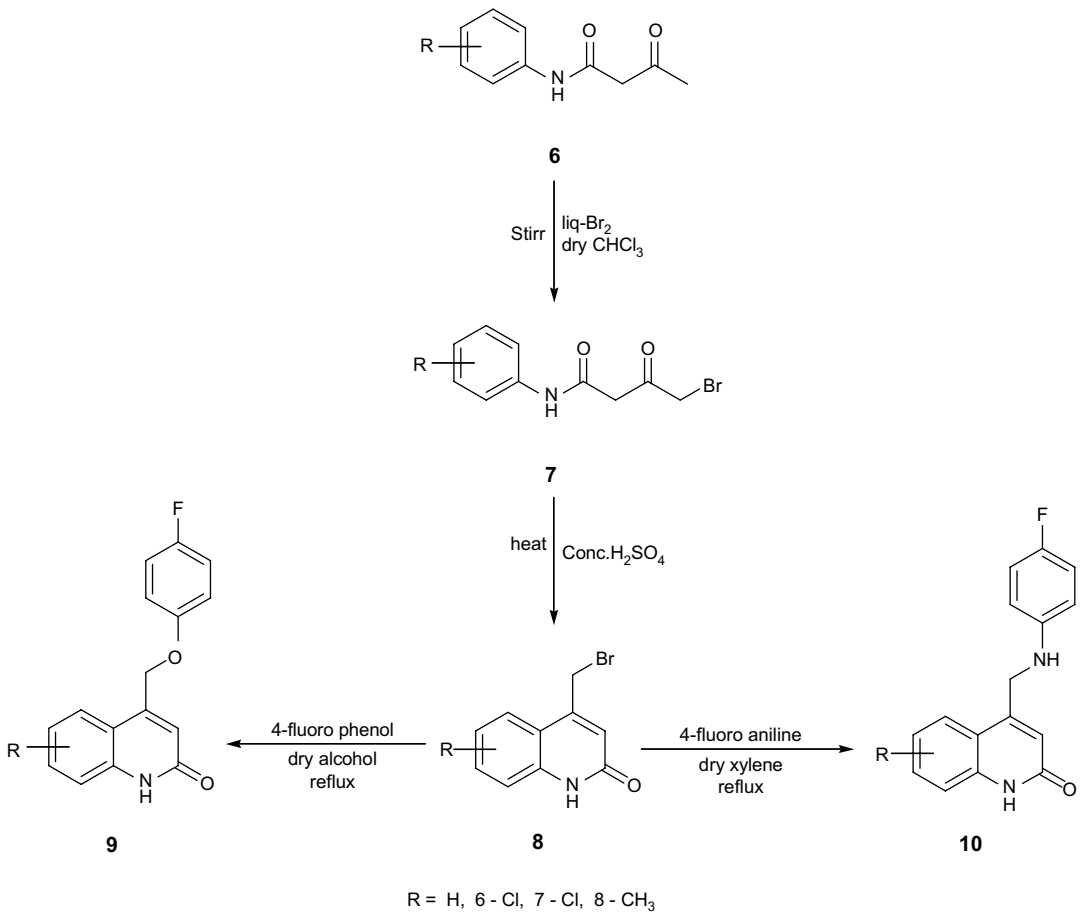
## 2. Chemistry

Synthesis of various 4-bromomethyl coumarins [21] was brought about by the Pechman cyclisation of phenols with 4-bromoethylacetoacetate (Scheme 1). The substituted 4-[(4′-fluoro) phenoxymethyl]-coumarins (**4a–e**) were synthesized at room temperature by stirring the reaction mixture of 4-fluorophenol and anhydrous $K_2CO_3$ with various 4-bromomethyl coumarins (**3a–e**) in dry acetone. On the other hand the substituted 4-[(4′-fluoro) anilinomethyl]-coumarins (**5a–e**) were synthesized by refluxing the reaction mixture of 4-fluoroaniline and various 4-bromomethyl coumarins in dry xylene at 135–148 °C. Similarly by a different sequence of reactions, 4-bromomethyl carbostyrils (**8a–d**) were synthesized by the bromination of acetoacetanilides and cyclising the intermediate ω-bromo acetoacetanilides in sulphuric acid [22] (Scheme 2). The substituted 4-[(4′-fluoro) phenoxymethyl]-carbostyrils (**9a–d**) were synthesized by refluxing the reaction mixture of 4-fluorophenol and anhydrous $K_2CO_3$ with various 4-bromomethyl carbostyrils in absolute alcohol at 100 °C. In an analogous manner the substituted 4-[(4′-fluoro)-anilinomethyl]-carbostyrils (**10a–d**) were synthesized by refluxing the reaction mixture of 4-fluoroaniline and various 4-bromomethyl carbostyrils in dry xylene at 135–148 °C.

All the products gave satisfactory analytical and spectroscopic data, which are in full accordance with their assigned structures. The ORTEP diagram of one of the compounds, 7-methyl-4-[(4′-fluoro)-anilinomethyl]-coumarin (**5c**), is reported (Fig. 1). The various new compounds synthesized during the present investigation are listed in Table 1.




Scheme 1. Synthesis of new 4-fluoro-aryloxymethyl and aminomethyl coumarins.




Scheme 2. Synthesis of new 4-fluoro-aryloxymethyl and aminomethyl 1-aza coumarins.

## 3. Pharmacology

### 3.1. Anti-microbial assay

All the new fluorinated analogues of coumarin and 1-aza coumarin were tested for in-vitro anti-microbial activity by cup–plate method [23–25]. Two bacterial microorganisms *Escherischia coli* (Gram +ve) and *Bacillus cirrhosis* (Gram −ve) and the two fungal microorganisms *Aspirgillus niger* and *Rhizoctonia bataticola* were used. DMF was used as a solvent control and the standard drugs used were Norfloxacin and Griseofulvin. The tests were carried out at a concentration of 100 μg ml$^{-1}$, 50 μg ml$^{-1}$, and 25 μg ml$^{-1}$. After 48 h of incubation at 37 °C, the zone of inhibition was measured in millimetres. The percentage inhibition of test compounds was related to the standard whose zone of inhibition was taken as 100%.

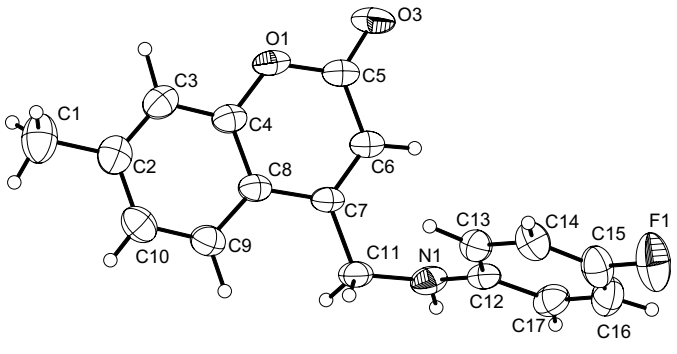



Fig. 1. ORTEP diagram of 7-methyl-4-[(4′-fluoro)-anilinomethyl]-coumarin.

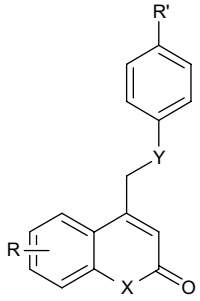
### 3.2. Acute toxicity

Groups of six albino mice, weighing 20–25 g were fasted overnight and treated per orally and intraperitoneally with the test compounds [26,27]. The dosage was varied from 1000 mg kg$^{-1}$ to 100 mg kg$^{-1}$ body weights. The animals were observed for 24 h for any signs of acute toxicity such as increased or decreased motor activity, tremors, convulsion, sedation, lacrimation etc. No mortality of the animals was observed even after 24 h. Hence the $LD_{50}$ cut off value of the test compounds was fixed as 1000 mg kg$^{-1}$, so that 100 mg kg$^{-1}$ i.e., 1/10 of cut off value was taken as screening dose for the evaluation of anti-inflammatory activity.

#### 3.2.1. Ulcerogenic activity

Male Wistar rats were fasted for 36 h. Indomethacin suspended in 1% CMC was given orally at a dose level of 20 mg kg$^{-1}$ (2 × 10 mg kg$^{-1}$) body weight and the test

Table 1
List of the new fluorinated coumarins and carbostyrils synthesized (**4a–10d**)




**(4a-10d)**

| Sl. no. | Compounds | R | R′ | X | Y |
|---|---|---|---|---|---|
| 1 | **4a** | 6-Cl | 4′-F | O | O |
| 2 | **4b** | 6-$CH_3$ | 4′-F | O | O |
| 3 | **4c** | 7-$CH_3$ | 4′-F | O | O |
| 4 | **4c1** | 7-$CH_3$ | 4′-Cl | O | O |
| 5 | **4c2** | 7-$CH_3$ | 4′-Br | O | O |
| 6 | **4d** | 5,6-Benz | 4′-F | O | O |
| 7 | **4e** | 7,8-Benz | 4′-F | O | O |
| 8 | **5a** | 6-Cl | 4′-F | O | NH |
| 9 | **5b** | 6-$CH_3$ | 4′-F | O | NH |
| 10 | **5c** | 7-$CH_3$ | 4′-F | O | NH |
| 11 | **5c1** | 7-$CH_3$ | 4′-Cl | O | NH |
| 12 | **5c2** | 7-$CH_3$ | 4′-Br | O | NH |
| 13 | **5d** | 5,6-Benz | 4′-F | O | NH |
| 14 | **5e** | 7,8-Benz | 4′-F | O | NH |
| 15 | **9a** | H | 4′-F | NH | O |
| 16 | **9b** | 6-Cl | 4′-F | NH | O |
| 17 | **9b1** | 6-Cl | 4′-Cl | NH | O |
| 18 | **9b2** | 6-Cl | 4′-Br | NH | O |
| 19 | **9c** | 7-Cl | 4′-F | NH | O |
| 20 | **9c1** | 7-Cl | 4′-Cl | NH | O |
| 21 | **9c2** | 7-Cl | 4′-Br | NH | O |
| 22 | **9d** | 8-$CH_3$ | 4′-F | NH | O |
| 23 | **9d1** | 8-$CH_3$ | 4′-Cl | NH | O |
| 24 | **9d2** | 8-$CH_3$ | 4′-Br | NH | O |
| 25 | **10a** | H | 4′-F | NH | NH |
| 26 | **10b** | 6-Cl | 4′-F | NH | NH |
| 27 | **10c** | 7-Cl | 4′-F | NH | NH |
| 28 | **10d** | 8-$CH_3$ | 4′-F | NH | NH |

compounds were administered twice at 2 h interval at a dose level of 400 mg $kg^{-1}$ body weight. The animals were sacrificed after 4 h and stomach was examined for lesions. The compounds showed low or no harmful effects. Indomethacin at lower doses produced serious gastric ulcers in all animals [28].

### 3.2.2. Analgesic activity

The analgesic activity of the test compounds was determined in-vivo by using abdominal constriction test induced by acetic acid 0.6% (0.1 ml 10 $g^{-1}$) in mice [29,30]. Albino mice of both sexes (18–22 g) were used. Compounds were administered orally (10 mg $kg^{-1}$) as a suspension in 5% carboxy methyl cellulose (vehicle). Indomethacin (10 mg $kg^{-1}$) was used as the standard drug under the same conditions.

### 3.2.3. Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was determined by formalin induced rat-paw oedema inhibition method according to Winter et al. [9,31], by employing 3.5% of formalin as the phlogistic agent. All the test compounds were administered orally as suspensions in 2% CMC, 30 min before the injection of the phlogistic agent, at a dose level of 100 mg $kg^{-1}$ body weight. Indomethacin was used as a standard at a dose level of 10 mg $kg^{-1}$ body weight. Groups of six Wistar rats of either sex were used in each experiment. Plain CMC (2%) served as a control. The paw oedema volume was measured with the help of plethysmograph by mercury displacement method at 0 h (immediately after injection of formalin), 1 h, 2 h, 3 h, 4 h and 5 h.

## 4. Results and discussion

### 4.1. Anti-microbial activity

The standard cup–plate method [23–25] was adopted for the determination of anti-bacterial and anti-fungal activities of the new fluorinated coumarins and the corresponding 1-aza coumarins.

The results of the anti-microbial activities of coumarin derivatives (Table 2) show that 4-aryloxymethylcoumarins **4c**, **4c1** and **4c2** with 7-$CH_3$ substitution in coumarin ring and F, Cl, Br at 4-position of the aryloxymethyl moiety, have shown up to 60% activity against both the bacterial strains and fungal strains at 100 μg $ml^{-1}$ concentration as compared to the standard. But at 25 μg $ml^{-1}$ concentration the activity is less than 20%. Hence further dilution was not taken up. Even in the case of **5c**, **5c1** and **5c2** with 7-$CH_3$ group in coumarin ring and F, Cl, and Br at 4-position of the arylaminomethyl moiety, a maximum of 70% activity was observed against both the bacterial and fungal strains at 100 μg $ml^{-1}$ concentration and up to 20% activity at 25 μg $ml^{-1}$ concentration as compared to the standard. Therefore only fluorinated aryloxymethyl and arylaminomethyl coumarins were used for further studies. Compounds **4a** and **5a** have shown more than 90% activity for both the bacterial and fungal strains at 100 μg $ml^{-1}$ concentration as compared to the standard. They were active up to 60% even at 25 μg $ml^{-1}$ concentration. In general the 4-aryloxymethyl and arylaminomethyl coumarin derivatives showed stronger anti-bacterial activity, even at the 25 μg $ml^{-1}$ concentration and presence of fluorine at 4-position of both aryloxymethyl and arylaminomethyl coumarin derivatives showed better anti-microbial activity as compared to the corresponding 4-chloro and 4-bromo derivatives.

Similarly in the anti-microbial activities of 1-aza coumarin derivatives (Table 3), it was observed that the 4-aryloxymethyl-1-aza coumarin **9b**, with 6-Cl substitution showed 81% growth inhibition for both bacterial and fungal strains at 100 μg $ml^{-1}$ concentration as compared to 50–60% activity of the corresponding chloro and bromo substituents in the aryloxy moiety (**9b1** and **9b2**, respectively). Further it was observed that compound **9b** retained up to 45% activity even at 25 μg $ml^{-1}$ concentration, whereas the other two derivatives were inactive at that concentration. Similarly **9c** with 7-Cl and 4′-F substitution showed about 78% anti-bacterial activity and

Table 2
Anti-bacterial and anti-fungal activities of selected coumarins

| Compounds | R | R′ | Zone of inhibition mm, % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Bacteria | | | | | | Fungi | | | | | |
| | | | *E. coli* Concentration, μg/ml | | | *B. cirrhosis* Concentration, μg/ml | | | *A. niger* Concentration, μg/ml | | | *R. bataticola* Concentration, μg/ml | | |
| | | | 100 μg | 50 μg | 25 μg | 100 μg | 50 μg | 25 μg | 100 μg | 50 μg | 25 μg | 100 μg | 50 μg | 25 μg |
| **4a** | 6-Cl | 4′-F | 238, 94 | 135, 60 | 92, 51 | 234, 97 | 148, 71 | 110, 64 | 216, 89 | 148, 71 | 106, 61 | 234, 89 | 168, 74 | 118, 65 |
| **4c** | 7-$CH_3$ | 4′-F | 164, 62 | 92, 37 | 44, 17 | 162, 64 | 88, 37 | 50, 21 | 136, 52 | 58, 21 | Nil, – | 148, 53 | 80, 30 | Nil, – |
| **4c1** | 7-$CH_3$ | 4′-Cl | 170, 65 | 88, 35 | 40, 14 | 158, 62 | 84, 33 | 40, 14 | 130, 50 | 60, 22 | Nil, – | 150, 54 | 75, 27 | Nil, – |
| **4c2** | 7-$CH_3$ | 4′-Br | 172, 66 | 92, 37 | 48, 20 | 160, 63 | 88, 37 | 48, 20 | 138, 52 | 60, 22 | Nil, – | 158, 57 | 84, 32 | Nil, – |
| **5a** | 6-Cl | 4′-F | 240, 95 | 142, 64 | 92, 51 | 238, 99 | 150, 83 | 110, 64 | 220, 90 | 152, 73 | 110, 64 | 240, 91 | 172, 76 | 122, 68 |
| **5c1** | 7-$CH_3$ | 4′-F | 188, 73 | 108, 46 | 58, 27 | 184, 74 | 120, 55 | 60, 28 | 168, 67 | 80, 33 | 40, 14 | 188, 70 | 118, 49 | 60, 26 |
| **5c2** | 7-$CH_3$ | 4′-Cl | 164, 62 | 90, 36 | 44, 17 | 152, 60 | 82, 34 | 44, 17 | 138, 52 | 58, 21 | Nil, – | 150, 54 | 84, 32 | Nil, – |
| **5c3** | 7-$CH_3$ | 4′-Br | 168, 64 | 88, 35 | 38, 13 | 160, 63 | 88, 37 | 50, 21 | 142, 55 | 62, 23 | Nil, – | 162, 59 | 90, 35 | Nil, – |
| Standard | – | – | 250 | 210 | 160 | 240 | 200 | 160 | 240 | 200 | 160 | 260 | 220 | 170 |
| Control | – | – | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

Standards used – Norfloxacin, Gresofulvin – 100% inhibition at each concentration.
Control – dimethyl formamide.

higher anti-fungal activity, 85%, as compared to the 50–60% activity of the corresponding chloro and bromo compounds **9c1** and **9c2**. In the case of 4-anilinomethyl-1-aza coumarins, **10b** and **10c**, with 6-Cl and 7-Cl substituents, the compounds have shown more than 75% activity against both the bacterial and fungal strains, at 100 μg $ml^{-1}$ concentration as compared to the standard. In general the fluorinated derivatives of 4-aryloxymethyl and arylaminomethyl 1-aza coumarins showed excellent anti-bacterial and anti-fungal activities up to 85% as compared to the corresponding 4-chloro and 4-bromo derivatives. At lower concentrations coumarins were found to be more active than 1-aza coumarins.

The graphical representation of anti-microbial activities of selected coumarins and 1-aza coumarins at 100 μg $ml^{-1}$ concentration is depicted in Fig. 2.

### 4.2. Acute toxicity studies

The acute toxicity studies of the test compounds (Table 4) were performed on albino mice fasted for 24 h. The animals were watched for mortality and symptoms until eighth day [26,27]. It is found that all the compounds possess good safety profile till the highest dose. No mortality of animals was observed even after 24 h.

### 4.3. Ulcerogenic activity

The ulcer index of the test compounds (Table 4) showed no harmful effects on the stomach, at the dose of 400 mg $kg^{-1}$ p.o, in fasted rats. Indomethacin at lower doses produced serious gastric ulcers in all animals [28].

### 4.4. Analgesic activity

Abdominal constriction response induced by acetic acid is a sensitive procedure to establish efficacy of peripherally acting analgesics. Intraperitoneal administration of acetic acid causes increase in the level of PGE2 and PGF 2α [29,30]. The results of analgesic activity (Table 4) indicate that compounds **5e** and **4b** showed highest analgesic activity of 76%

Table 3
Anti-bacterial and anti-fungal activities of selected 1-aza coumarins

| Compounds | R | R′ | Zone of inhibition mm, % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Bacteria | | | | | | Fungi | | | | | |
| | | | *E. coli* Concentration, μg/ml | | | *B. cirrhosis* Concentration, μg/ml | | | *A. niger* Concentration, μg/ml | | | *R. bataticola* Concentration, μg/ml | | |
| | | | 100 μg | 50 μg | 25 μg | 100 μg | 50 μg | 25 μg | 100 μg | 50 μg | 25 μg | 100 μg | 50 μg | 25 μg |
| **9b** | 6-Cl | 4′-F | 216, 81 | 144, 62 | 88, 45 | 204, 80 | 118, 51 | 60, 28 | 186, 75 | 104, 44 | 62, 29 | 222, 84 | 150, 68 | 90, 50 |
| **9b1** | 6-Cl | 4′-Cl | 142, 50 | 80, 30 | Nil, – | 158, 60 | 80, 31 | Nil, – | 138, 53 | 68, 25 | Nil, – | 152, 55 | 84, 33 | Nil, – |
| **9b2** | 6-Cl | 4′-Br | 140, 50 | 82, 30 | Nil, – | 154, 58 | 68, 25 | Nil, – | 158, 62 | 90, 36 | 50, 21 | 152, 55 | 82, 32 | Nil, – |
| **9c** | 7-Cl | 4′-F | 209, 78 | 132, 56 | 72, 34 | 196, 76 | 96, 40 | 52, 22 | 194, 79 | 110, 47 | 68, 34 | 228, 86 | 158, 72 | 98, 55 |
| **9c1** | 7-Cl | 4′-Cl | 136, 48 | 76, 27 | Nil, – | 150, 56 | 68, 25 | Nil, – | 132, 50 | 60, 21 | Nil, – | 144, 51 | 78, 35 | Nil, – |
| **9c2** | 7-Cl | 4′-Br | 140, 50 | 80, 30 | Nil, – | 158, 60 | 70, 26 | Nil, – | 154, 60 | 88, 35 | 46, 18 | 150, 54 | 80, 31 | Nil, – |
| **9d** | 8-$CH_3$ | 4′-F | 158, 57 | 86, 33 | Nil, – | 164, 62 | 80, 31 | Nil, – | 154, 60 | 90, 36 | 52, 22 | 160, 58 | 90, 36 | 50, 21 |
| **10a** | H | 4′-F | 158, 57 | 92, 34 | Nil, – | 166, 68 | 88, 36 | Nil, – | 160, 63 | 98, 41 | 54, 24 | 164, 60 | 90, 36 | 50, 21 |
| **10b** | 6-Cl | 4′-F | 188, 70 | 120, 50 | 60, 26 | 196, 76 | 98, 41 | 52, 22 | 190, 77 | 110, 47 | 70, 35 | 200, 75 | 128, 56 | 82, 44 |
| **10c** | 7-Cl | 4′-F | 196, 73 | 128, 54 | 68, 32 | 200, 78 | 104, 44 | 58, 27 | 198, 80 | 118, 52 | 78, 41 | 210, 79 | 138, 62 | 88, 48 |
| Standard | – | – | 260 | 220 | 170 | 250 | 210 | 160 | 240 | 210 | 160 | 260 | 210 | 160 |
| Control | – | – | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

Standards used – Norfloxacin, Gresofulvin – 100% inhibition at each concentration.
Control – dimethyl formamide.

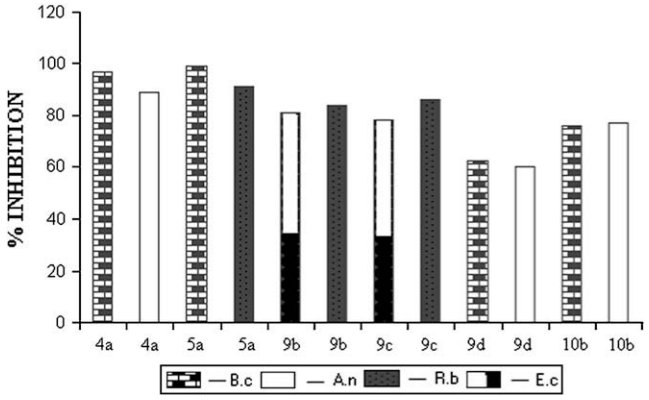



Fig. 2. The anti-microbial activities of selected fluoro coumarins and 1-aza coumarins. Columns represent the % inhibition of selected compounds for bacteria and fungi at 100 μg ml$^{-1}$ concentration. B.c (*B. cirrhosis*), A.n (*A. niger*), R.b (*R. bataticola*), E.c (*E. coli*).

and 72%, respectively. Compounds **10d**, **9c** and **4a** showed 66%, 61% and 56% inhibition of writhing, respectively, higher than the standard and most of the compounds showed moderate analgesic activity when compared to the control and the standard group.

### 4.5. Anti-inflammatory activity

The anti-inflammatory activity of the test compounds was determined by formalin induced rat-paw oedema method [9,31]. The results of anti-inflammatory activity (Table 5) indicate that most of the compounds showed an extremely significant anti-inflammatory activity when compared to the control and the standard group. Coumarin derivatives **5c**, **5e** and **4a** showed maximum of 83%, 81% and 80% inhibition of inflammation, respectively, at the end of the fifth hour. Similarly 1-aza coumarin derivatives **10d** and **9b** showed 85% and 80% inhibition of inflammation, respectively, at the end of fifth hour and the activity was as good as the standard and it is interesting to note that the presence of fluorine at 4′-position induces better activity than the other halogenated compounds.

Table 4
Analgesic activities, ulcerogenic and acute toxicity studies of the test compounds

| Compounds | Analgesic activity,[a] % inhibition of writhing | Ulcerogenic score,[b] 400 mg kg$^{-1}$ | Acute toxicity LD$_{50}$, mg kg$^{-1}$ | |
|---|---|---|---|---|
| | | | i.p | p.o |
| **4a** | 56.21 | 66.66 | >700 | >1000 |
| **4b** | 72.72 | 57.29 | >700 | >1000 |
| **4e** | 23.18 | 61.80 | >800 | >1000 |
| **5c** | 3.96 | 64.93 | >800 | >1000 |
| **5d** | 10.5 | 64.93 | >800 | >1000 |
| **5e** | 76.42 | 64.93 | >700 | >1000 |
| **9a** | 15.84 | 60.71 | >800 | >1000 |
| **9b** | 26.9 | 71.42 | >800 | >1000 |
| **9c** | 61.27 | 67.5 | >800 | >1000 |
| **10b** | 7.99 | 58.57 | >700 | >1000 |
| **10c** | 28.57 | 61.42 | >700 | >1000 |
| **10d** | 66.97 | 55.35 | >700 | >1000 |
| Standard | 46.22 | 185.14 | ~15 | ~25 |
| Control | – | – | – | – |

Oral administration for all the test compounds at a dose level of 10 mg kg$^{-1}$ body weight.
[a] Standard used – Indomethacin – 10 mg kg$^{-1}$.
[b] Standard used – Indomethacin – 2 × 10 mg kg$^{-1}$.

## 5. Conclusion

The present study has shown that the introduction of fluorine at the 4′-position in the aryloxy and arylamino moieties of both coumarin and 1-aza-coumarin enhances the anti-microbial as well as analgesic and anti-inflammatory activities, which might serve as new templates in the synthesis and development of potent therapeutics. Our findings will have a good impact on chemists and biochemists for further investigations in this field in search of fluorine containing anti-microbial, analgesic and anti-inflammatory agents.

## 6. Experimental protocol

The melting points of the products were determined by open capillaries on a Buchi apparatus and are uncorrected. The IR spectra were recorded on a Nicolet Impact-410 FT-IR Spectrophotometer, using KBr pellets. $^{1}$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AC-300F 300 MHz spectrometer in $CDCl_3$ using TMS as an internal standard with $^{1}$H resonant frequency of 300 MHz and $^{13}$C resonant frequency of 75 MHz. $D_2O$ exchange was applied to confirm the assignment of the signals of NH protons. $^{19}$F NMR spectra were obtained on a Bruker AV 500 MHz spectrometer using $CF_3COOH$ (TFA) as an external standard, positive for downfield shift with $^{19}$F resonant frequency of 470 MHz. Mass spectra were recorded on an Autospec EI-MS and FAB-MS. Elemental analysis was carried out by using Heraus CHN rapid analyzer. All the compounds gave C, H and N analysis within ±0.4% of the theoretical values. The homogeneity of the compounds was described by TLC on aluminum silica gel 60 $F_{254}$ (Merck) detected by UV light (254 nm) and iodine vapours. The reagents were all of analytical reagent-grade or chemically pure. All solvents were dried, deoxygenated and redistilled before use.

### 6.1. General procedure for the preparation of compounds *4a–e*

A mixture of substituted 4-bromomethyl coumarins (**3a–e**) (4.0 mmol), 4-fluoro-phenol (4.0 mmol) and powdered anhydrous potassium carbonate (4.0 mmol) in super dry acetone (20 ml) was stirred at room temperature for 16 h; after completion of the reaction, the separated solid was filtered, washed with 20% HCl and with excess of cold water, dried and crystallized from suitable solvent.

Table 5
Anti-inflammatory activities of the test compounds (formalin induced rat-paw oedema method)

| Compounds | Anti-inflammatory activity | | | | |
|---|---|---|---|---|---|
| | Oedema volume at different time intervals ± SEM (% inhibition) | | | | |
| | 1 h | 2 h | 3 h | 4 h | 5 h |
| **4a** | 0.48 ± 0.04 (39) | 0.87 ± 0.04 (38) | 0.77 ± 0.05 (58) | 0.55 ± 0.08 (71) | 0.4 ± 0.09 (80) |
| **4b** | 0.45 ± 0.07 (40) | 0.89 ± 0.11 (36) | 0.95 ± 0.1 (48) | 0.68 ± 0.04 (64) | 0.48 ± 0.04 (76) |
| **4e** | 0.43 ± 0.06 (46) | 0.98 ± 0.08 (30) | 1.0 ± 0.06 (45) | 0.76 ± 0.05 (66) | 0.51 ± 0.06 (75) |
| **5c** | 0.30 ± 0.03 (62) | 0.88 ± 0.07 (37) | 0.83 ± 0.08 (54) | 0.64 ± 0.08 (67) | 0.35 ± 0.04 (83) |
| **5d** | 0.29 ± 0.03 (63) | 0.92 ± 0.04 (35) | 0.85 ± 0.07 (53) | 0.64 ± 0.08 (60) | 0.46 ± 0.06 (77) |
| **5e** | 0.15 ± 0.02 (81) | 0.64 ± 0.06 (54) | 0.86 ± 0.07 (53) | 0.56 ± 0.09 (71) | 0.38 ± 0.08 (81) |
| **9a** | 0.55 ± 0.07 (35) | 1.11 ± 0.06 (23) | 0.88 ± 0.04 (52) | 0.71 ± 0.06 (63) | 0.46 ± 0.03 (77) |
| **9b** | 0.48 ± 0.04 (39) | 1.01 ± 0.07 (28) | 0.78 ± 0.08 (57) | 0.55 ± 0.08 (71) | 0.4 ± 0.06 (80) |
| **9c** | 0.6 ± 0.02 (24) | 1.06 ± 0.03 (24) | 0.83 ± 0.04 (54) | 0.65 ± 0.02 (66) | 0.45 ± 0.03 (78) |
| **10b** | 0.72 ± 0.03 (9) | 1.23 ± 0.05 (12) | 0.98 ± 0.03 (46) | 0.78 ± 0.04 (59) | 0.46 ± 0.03 (77) |
| **10c** | 0.76 ± 0.04 (4) | 1.35 ± 0.05 (4) | 1.06 ± 0.08 (42) | 0.81 ± 0.08 (57) | 0.46 ± 0.06 (77) |
| **10d** | 0.61 ± 0.04 (23) | 1.01 ± 0.04 (28) | 1.71 ± 0.06 (6) | 0.51 ± 0.07 (73) | 0.31 ± 0.07 (85) |
| Standard | 0.51 ± 0.03 (35) | 0.8 ± 0.05 (43) | 0.55 ± 0.05 (75) | 0.46 ± 0.06 (76) | 0.3 ± 0.02 (85) |
| Control | 0.79 ± 0.010 (0) | 1.40 ± 0.013 (0) | 1.82 ± 0.013 (0) | 1.91 ± 0.011 (0) | 2.02 ± 0.01 (0) |

Oral administration of the test compounds, at a dose level of 100 mg $kg^{-1}$ body weight.
Standard used − Indomethacin − 10 mg $kg^{-1}$.
The data are analyzed by one-way ANOVA followed by Tukey comparison of all pairs.
Model − acute inflammation.
Method − formalin induced.

*6.1.1. 6-Chloro-4-[(4′-fluoro) phenoxymethyl]-coumarin (**4a**)*

Yield: 72%; light green crystals (ethanol); m.p. 205–207 °C; IR (KBr): 1728, 1150 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.40 (s, 2H, C4−$CH_2$), 6.30 (s, 1H, C3−H), 6.50–7.80 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz $CDCl_3$, δ ppm): 161.50, 158.28, 156.34, 154.28, 136.89, 133.48, 132.80, 127.08, 124.60, 119.40, 116.30, 116.21, 114.47, 114.30, 109.10, 65.98; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −123.62 (s, 1F, C4′−F); EI-MS: *m/z* 304 ($M^+$). Anal. Calc. for $C_{16}H_{10}ClFO_3$: C, 63.07; H, 3.31; found: C, 62.87; H, 3.61%.

*6.1.2. 6-Methyl-4-[(4′-fluoro) phenoxymethyl]-coumarin (**4b**)*

Yield: 85%; colorless crystals (ethanol + dioxan); m.p. 188–190 °C; IR (KBr): 1733, 1145 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.45 (s, 3H, C6−$CH_3$), 5.21 (s, 2H, C4−$CH_2$), 6.67 (s, 1H, C3−H), 6.95–7.41 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.18, 159.04, 156.80, 155.50, 151.08, 138.70, 127.03, 126.90, 124.24, 122.17, 116.50, 116.41, 114.44, 114.20, 109.20, 65.30, 18.90; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −124.34 (s, 1F, C4′−F); EI-MS: *m/z* 284 ($M^+$). Anal. Calc. for $C_{17}H_{13}FO_3$: C, 71.80; H, 4.57; found: C, 71.46; H, 4.29%.

*6.1.3. 7-Methyl-4-[(4′-fluoro) phenoxymethyl]-coumarin (**4c**)*

Yield: 80%; colorless crystals (ethanol + dioxan); m.p. 194–196 °C; IR (KBr): 1720, 1140 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.48 (s, 3H, C7−$CH_3$), 5.20 (s, 2H, C4−$CH_2$), 6.45 (s, 1H, C3−H), 6.52–7.63 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.30, 159.20, 156.77, 155.24, 151.10, 137.78, 126.89, 126.54, 124.30, 122.80, 116.14, 116.10, 114.23, 114.04, 109.17, 65.44, 19.00; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −125.82 (s, 1F, C4′−F); EI-MS: *m/z* 284 ($M^+$). Anal. Calc. for $C_{17}H_{13}FO_3$: C, 71.80; H, 4.58; found: C, 71.40; H, 4.24%.

*6.1.4. 7-Methyl-4-[(4′-chloro) phenoxymethyl]-coumarin (**4c1**)*

Yield: 78%; colorless crystals (ethanol + dioxan); m.p. 181–183 °C; IR (KBr): 1701, 3032, 1152 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.45 (s, 3H, C7−$CH_3$), 5.22 (s, 2H, C4−$CH_2$), 6.55 (s, 1H, C3−H), 6.94–7.41 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 161.38, 159.24, 156.77, 155.24, 151.10, 137.80, 127.89, 126.74, 124.30, 122.88, 116.14, 116.18, 114.23, 114.04, 109.17, 65.48, 18.00; EI-MS: *m/z* 300 ($M^+$). Anal. Calc. for $C_{17}H_{13}ClO_3$: C, 68.00; H, 4.33; found: C, 67.80; H, 4.24%.

*6.1.5. 7-Methyl-4-[(4′-bromo) phenoxymethyl]-coumarin (**4c2**)*

Yield: 80%; colorless crystals (ethanol + dioxan); m.p. 176–178 °C; IR (KBr): 1708, 3040, 1146 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.38 (s, 3H, C7−$CH_3$), 5.30 (s, 2H, C4−$CH_2$), 6.48 (s, 1H, C3−H), 6.92–7.48 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.50, 158.90, 156.80, 155.24, 151.10, 138.80, 127.89, 126.74, 124.30, 122.88, 116.14, 116.20, 114.23, 114.04, 109.17, 65.48, 19.04; EI-MS: *m/z* 345 ($M^+$). Anal. Calc. for $C_{17}H_{13}BrO_3$: C, 59.31; H, 3.76; found: C, 59.09; H, 3.50%.

*6.1.6. 5,6-Benzo-4-[(4′-fluoro) phenoxymethyl]-coumarin (**4d**)*

Yield: 86%; pale yellow crystals (ethanol + dioxan); m.p. 168–170 °C; IR (KBr): 1717, 1147 $cm^{-1}$; $^1H$ NMR

(300 MHz, $CDCl_3$, δ ppm): 5.38 (s, 2H, C4−$CH_2$), 6.64 (s, 1H, C3−H), 6.94−8.50 (m, 10H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.08, 159.78, 158.51, 153.80, 148.21, 133.40, 132.70, 127.06, 123.60, 122.34, 121.80, 120.41, 119.40, 118.28, 116.50, 116.31, 114.70, 114.56, 109.28, 66.04; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −122.61 (s, 1F, C4′−F); EI-MS: *m/z* 320 ($M^+$). Anal. Calc. for $C_{20}H_{13}FO_3$: C, 74.99; H, 4.09; found: C, 74.50; H, 3.80%.

### *6.1.7. 7,8-Benzo-4-[(4′-fluoro) phenoxymethyl]-coumarin (**4e**)*

Yield: 88%; light green crystals (ethanol); m.p. 240−242 °C; IR (KBr): 1702, 1161 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.25 (s, 2H, C4−$CH_2$), 6.45 (s, 1H, C3−H), 6.90−8.22 (m, 10H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 163.10, 159.08, 158.16, 153.20, 146.81, 133.14, 132.70, 128.44, 126.40, 126.20, 124.17, 122.80, 121.08, 119.80, 116.54, 116.38, 114.70, 112.30, 108.80, 66.21; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −128.69 (s, 1F, C4′−F); EI-MS: *m/z* 320 ($M^+$). Anal. Calc. for $C_{20}H_{13}FO_3$: C, 74.99; H, 4.09; found: C, 74.55; H, 3.92%.

## *6.2. General procedure for the preparation of compounds **5a−e***

A mixture of substituted 4-bromomethyl coumarins (**3a−e**) (4.0 mmol) and 4-fluoro-aniline (4.0 mmol) in super dry xylene (20 ml) was refluxed on an oil bath for 8 h (135−148 °C). After the completion of the reaction, the separated solid was filtered, washed with excess of cold ethanol, dried and crystallized from suitable solvent.

### *6.2.1. 6-Chloro-4-[(4′-fluoro) anilinomethyl]-coumarin (**5a**)*

Yield: 78%; colorless crystals (ethanol + dioxan); m.p. 238−240 °C; IR (KBr): 1709, 3376, 1184 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 4.14 (s, 1H, $CH_2NH$), 4.56 (s, 2H, C4−$CH_2$), 6.30 (s, 1H, C3−H), 6.50−8.90 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 161.48, 159.14, 156.66, 150.30, 138.92, 131.26, 129.50, 128.80, 127.17, 122.80, 116.30, 116.21, 113.98, 112.20, 108.76, 62.70; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −124.83 (s, 1F, C4′−F); EI-MS: *m/z* 303 ($M^+$). Anal. Calc. for $C_{16}H_{11}ClFNO_2$: C, 63.27; H, 3.65; N, 4.61; found: C, 63.36; H, 3.38; N, 4.50%.

### *6.2.2. 6-Methyl-4-[(4′-fluoro) anilinomethyl]-coumarin (**5b**)*

Yield: 82%; colorless crystals (ethanol + dioxan); m.p. 215−217 °C; IR (KBr): 1702, 3369, 1166 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.45 (s, 3H, C6−$CH_3$), 4.15 (s, 1H, $CH_2NH$), 4.50 (s, 2H, C4−$CH_2$), 6.32 (s, 1H, C3−H), 6.58−8.40 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.14, 158.20, 156.48, 148.31, 140.93, 134.50, 128.17, 128.10, 126.30, 121.41, 116.31, 116.20, 112.90, 112.81, 109.40, 63.10, 20.13; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −123.82 (s, 1F, C4′−F); EI-MS: *m/z* 283 ($M^+$). Anal. Calc. for $C_{17}H_{14}FNO_2$: C, 72.07; H, 4.98; N, 4.94; found: C, 71.81; H, 4.60; N, 4.78%.

### *6.2.3. 7-Methyl-4-[(4′-fluoro) anilinomethyl]-coumarin (**5c**)*

Yield: 88%; colorless crystals (ethanol); m.p. 141−143 °C; IR (KBr): 1718, 3348, 1145.1 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.47 (s, 3H, C7−$CH_3$), 4.17 (s, 1H, $CH_2NH$), 4.50 (s, 2H, C4−$CH_2$), 6.25 (s, 1H, C3−H), 6.48−9.02 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.40, 159.30, 157.03, 149.29, 142.30, 134.60, 129.07, 128.90, 126.14, 121.14, 116.12, 116.03, 112.70, 112.61, 109.20, 63.18, 19.83; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −126.64 (s, 1F, C4′−F); EI-MS: *m/z* 283 ($M^+$). Anal. Calc. for $C_{17}H_{14}FNO_2$: C, 72.07; H, 4.98; N, 4.94; found: C, 71.88; H, 4.54; N, 4.79%.

### *6.2.4. 7-Methyl-4-[(4′-chloro) anilinomethyl]-coumarin (**5c1**)*

Yield: 76%; colorless crystals (ethanol + dioxan); m.p. 200−202 °C; IR (KBr): 3342, 1725, 1142 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.38 (s, 3H, C7−$CH_3$), 4.10 (s, 1H, $CH_2NH$), 4.48 (s, 2H, C4−$CH_2$), 6.28 (s, 1H, C3−H), 6.50−9.10 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 161.80, 159.58, 158.00, 149.24, 142.30, 134.60, 129.07, 128.90, 126.14, 121.14, 116.12, 116.00, 112.70, 112.61, 109.28, 63.34, 18.90; EI-MS: *m/z* 299 ($M^+$). Anal. Calc. for $C_{17}H_{14}ClNO_2$: C, 68.22; H, 4.68; N, 4.68; found: C, 68.00; H, 4.54; N, 4.43%.

### *6.2.5. 7-Methyl-4-[(4′-bromo) anilinomethyl]-coumarin (**5c2**)*

Yield: 80%; colorless crystals (ethanol + dioxan); m.p. 230−232 °C; IR (KBr): 3350, 1712, 1158 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.42 (s, 3H, C7−$CH_3$), 4.10 (s, 1H, $CH_2NH$), 4.62 (s, 2H, C4−$CH_2$), 6.38 (s, 1H, C3−H), 6.48−9.08 (m, 7H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.88, 159.00, 157.43, 149.29, 142.30, 134.60, 129.00, 128.90, 126.14, 121.14, 116.12, 116.03, 112.70, 112.61, 109.20, 63.00, 18.25; EI-MS: *m/z* 344 ($M^+$). Anal. Calc. for $C_{17}H_{14}BrNO_2$: C, 59.30; H, 4.06; N, 4.06; found: C, 59.18; H, 3.94; N, 3.83%.

### *6.2.6. 5,6-Benzo-4-[(4′-fluoro) anilinomethyl]-coumarin (**5d**)*

Yield: 92%; yellow crystals (ethanol + dioxan); m.p. 210−212 °C; IR (KBr): 1700, 3363, 1150 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 4.35 (s, 1H, $CH_2NH$), 4.92 (s, 2H, C4−$CH_2$), 6.43 (s, 1H, C3−H), 6.50−8.30 (m, 10H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 161.88, 158.60, 154.38, 151.71, 140.11, 131.68, 130.90, 128.20, 127.90, 126.12, 123.21, 118.13, 117.90, 116.28, 116.21, 113.22, 113.18, 112.80, 108.60, 62.76; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −122.64 (s, 1F, C4′−F); EI-MS: *m/z* 319 ($M^+$). Anal. Calc. for $C_{20}H_{14}FNO_2$: C, 75.22; H, 4.42; N, 4.39; found: C, 74.84; H, 4.10; N, 4.09%.

### *6.2.7. 7,8-Benzo-4-[(4′-fluoro) anilinomethyl]-coumarin (**5e**)*

Yield: 90%; yellow crystals (ethanol + dioxan); m.p. 202−204 °C; IR (KBr): 1720, 3338, 1140 $cm^{-1}$; $^1H$ NMR

(300 MHz, $CDCl_3$, δ ppm): 4.32 (s, 1H, $CH_2NH$), 4.90 (s, 2H, C4–$CH_2$), 6.46 (s, 1H, C3–H), 6.62–8.34 (m, 10H, Ar-H); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.10, 158.17, 155.14, 150.90, 141.34, 132.08, 130.87, 128.23, 127.80, 126.06, 125.94, 123.20, 119.20, 118.71, 116.10, 116.02, 113.71, 113.52, 108.14, 62.30; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −128.67 (s, 1F, C4′–F); EI-MS: *m/z* 319 ($M^+$). Anal. Calc. for $C_{20}H_{14}FNO_2$: C, 75.22; H, 4.42; N, 4.39; found: C, 74.90; H, 4.14; N, 4.20%.

### *6.3. General procedure for the preparation of compounds **9a–d***

A mixture of substituted 4-bromomethylcarbostyril (**8a–d**) (4.0 mmol), 4′-fluoro-phenol (4.0 mmol) and powdered anhydrous potassium carbonate (4.0 mmol) in super dry alcohol (20 ml) was refluxed on a water bath for 10 h. After the completion of the reaction, the separated solid was filtered, washed with 20% HCl and with excess of cold water, dried and crystallized from suitable solvent.

#### *6.3.1. 4-[(4′-Fluoro) phenoxymethyl]-1-aza coumarin (**9a**)*

Yield: 70%; colorless crystals (ethanol + dioxan); m.p. 230–232 °C; IR (KBr): 1665, 3431, 1140 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.32 (s, 2H, C4–$CH_2$), 6.95–7.88 (m, 9H, Ar-H), 12.37 (s, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 160.21, 158.51, 156.67, 153.67, 136.38, 133.78, 132.89, 126.15, 123.66, 118.38, 116.47, 116.38, 114.69, 114.58, 108.90, 66.74; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −122.94 (s, 1F, C4′–F); FAB-MS: *m/z* 270(M + H). Anal. Calc. for $C_{16}H_{12}FNO_2$: C, 71.37; H, 4.49; N, 5.20; found: C, 71.07; H, 4.80; N, 5.23%.

#### *6.3.2. 6-Chloro-4-[(4′-fluoro) phenoxymethyl]-1-aza coumarin (**9b**)*

Yield: 78%; colorless crystals (ethanol + dioxan); m.p. 240–242 °C; IR (KBr): 1672, 3440, 1130 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.28 (s, 2H, C4–$CH_2$), 6.35 (s, 1H, C3–H), 6.88–7.90 (m, 7H, Ar-H), 11.95 (s, 1H, NH); $^{13}C$ NMR ($CDCl_3$, δ ppm): 161.00, 159.34, 156.19, 151.87, 136.31, 132.74, 129.76, 127.31, 125.95, 123.61, 116.59, 116.19, 114.96, 114.84, 109.04, 66.32; $^{19}F$ NMR (300 MHz, $CDCl_3$, δ ppm): −123.68 (s, 1F, C4′–F); FAB-MS: *m/z* 304 (M + H). Anal. Calc. for $C_{16}H_{11}FClNO_2$: C, 63.27; H, 3.65; N, 4.61; found: C, 63.10; H, 3.80; N, 4.31%.

#### *6.3.3. 6-Chloro-4-[(4′-chloro) phenoxymethyl]-1-aza coumarin (**9b1**)*

Yield: 78%; colorless crystals (acetic acid); m.p. 250–252 °C; IR (KBr): 1660, 3434, 1140 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.48 (s, 2H, C4–$CH_2$), 6.70–7.92 (m, 8H, Ar-H), 11.88 (s, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 162.10, 159.44, 157.00, 151.87, 136.31, 132.74, 129.46, 127.31, 125.95, 123.61, 116.60, 116.19, 114.66, 114.84, 109.04, 66.40; FAB-MS: *m/z* 320 (M + H). Anal. Calc. for $C_{16}H_{11}Cl_2NO_2$: C, 60.18; H, 3.44; N, 4.38; found: C, 60.00; H, 3.24; N, 4.10%.

#### *6.3.4. 6-Chloro-4-[(4′-bromo) phenoxymethyl]-1-aza coumarin (**9b2**)*

Yield: 75%; colorless crystals (acetic acid); m.p. 270–272 °C; IR (KBr): 1655, 3419, 1147 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.45 (s, 2H, C4–$CH_2$), 6.88–7.95 (m, 8H, Ar-H), 11.86 (s, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 161.88, 159.48, 156.96, 151.87, 137.00, 132.74, 129.46, 128.08, 125.95, 123.61, 116.60, 116.20, 114.66, 114.80, 109.04, 65.88; FAB-MS: *m/z* 365 (M + H). Anal. Calc. for $C_{16}H_{11}ClBrNO_2$: C, 52.74; H, 3.02; N, 3.84; found: C, 52.50; H, 2.88; N, 3.61%.

#### *6.3.5. 7-Chloro-4-[(4′-fluoro) phenoxymethyl]-1-aza coumarin (**9c**)*

Yield: 72%; colorless crystals (ethanol + dioxan); m.p. 200–202 °C; IR (KBr): 1659, 3425, 1120 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.55 (s, 2H, C4–$CH_2$), 6.44 (s, 1H, C3–H), 6.95–8.00 (m, 7H, Ar-H), 11.88 (s, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 161.21, 158.40, 156.30, 151.17, 135.90, 132.60, 129.86, 128.00, 126.10, 123.60, 116.40, 116.20, 114.80, 114.74, 110.01, 66.21; $^{19}F$ NMR (470 MHz, $CDCl_3$, δ ppm): −123.21 (s, 1F, C4′–F); FAB-MS: *m/z* 304 (M + H). Anal. Calc. for $C_{16}H_{11}FClNO_2$: C, 63.27; H, 3.65; N, 4.61; found: C, 63.14; H, 3.78; N, 4.33%.

#### *6.3.6. 7-Chloro-4-[(4′-chloro) phenoxymethyl]-1-aza coumarin (**9c1**)*

Yield: 78%; colorless crystals (acetic acid); m.p. 230–232 °C; IR (KBr): 1658, 3428, 1128 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.35 (s, 2H, C4–$CH_2$), 6.94–7.76 (m, 7H, Ar-H), 11.85 (s, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 160.00, 158.32, 156.38, 151.20, 135.90, 132.60, 129.86, 128.09, 126.10, 123.60, 116.44, 116.20, 114.80, 114.74, 110.11, 66.18; FAB-MS: *m/z* 320 (M + H). Anal. Calc. for $C_{16}H_{11}Cl_2NO_2$: C, 60.18; H, 3.44; N, 4.38; found: C, 60.10; H, 3.18; N, 4.04%.

#### *6.3.7. 7-Chloro-4-[(4′-bromo) phenoxymethyl]-1-aza coumarin (**9c2**)*

Yield: 78%; colorless crystals (ethanol); m.p. 191–193 °C; IR (KBr): 1668, 3424, 1128 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 5.18 (s, 2H, C4–$CH_2$), 6.53–8.04 (m, 7H, Ar-H), 11.94 (s, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 161.22, 159.20, 156.38, 151.20, 135.90, 132.60, 128.66, 128.09, 126.10, 123.60, 116.44, 115.80, 114.80, 114.74, 110.11, 67.04; FAB-MS: *m/z* 365 (M + H). Anal. Calc. for $C_{16}H_{11}ClBrNO_2$: C, 52.74; H, 3.02; N, 3.84; found: C, 52.54; H, 2.78; N, 3.53%.

#### *6.3.8. 8-Methyl-4-[(4′-fluoro) phenoxymethyl]-1-aza coumarin (**9d**)*

Yield: 84%; colorless crystals (ethanol); m.p. 199–201 °C; IR (KBr): 1659, 3450, 1123 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$, δ ppm): 2.60 (s, 3H, C8–$CH_3$), 5.50 (s, 2H, C4–$CH_2$), 6.45 (s, 1H, C3–H), 6.98–7.98 (m, 7H, Ar-H), 11.80 (s, 1H, NH); $^{13}C$ NMR (75 MHz, $CDCl_3$, δ ppm): 161.53, 159.69, 156.51, 153.66, 134.79, 134.11, 126.68, 125.78,

121.54, 120.24, 116.40, 116.14, 112.80, 114.14, 108.89, 66.82, 16.93; $^{19}$F NMR (470 MHz, $CDCl_3$, $\delta$ ppm): −124.23 (s, 1F, C4′−F); FAB-MS: *m/z* 284 (M + H). Anal. Calc. for $C_{17}H_{14}FNO_2$: C, 72.07; H, 4.98; N, 4.94; found: C, 72.20; H, 4.54; N, 4.80%.

*6.3.9. 8-Methyl-4-[(4′-chloro) phenoxymethyl]-1-aza coumarin (**9d1**)*

Yield: 80%; colorless crystals (ethanol + dioxan); m.p. 236–238 °C; IR (KBr): 1660, 3445, 1129 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$, $\delta$ ppm): 2.58 (s, 3H, C8−$CH_3$), 5.52 (s, 2H, C4−$CH_2$), 6.92–7.95 (m, 8H, Ar-H), 11.78 (s, 1H, NH); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$ ppm): 160.43, 159.60, 157.00, 153.78, 134.79, 134.90, 126.68, 125.78, 121.54, 120.64, 116.40, 116.14, 112.52, 114.14, 108.89, 67.52, 18.05; FAB-MS: 300 (M + H). Anal. Calc. for $C_{17}H_{14}ClNO_2$: C, 68.22; H, 3.67; N, 4.68; found: C, 68.10; H, 3.44; N, 4.30%.

*6.3.10. 8-Methyl-4-[(4′-bromo) phenoxymethyl]-1-aza coumarin (**9d2**)*

Yield: 82%; colorless crystals (acetic acid); m.p. 238–240 °C; IR (KBr): 1672, 3429, 1122 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$, $\delta$ ppm): 2.48 (s, 3H, C8−$CH_3$), 5.62 (s, 2H, C4−$CH_2$), 6.76–7.90 (m, 8H, Ar-H), 11.72 (s, 1H, NH); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$ ppm): 160.40, 159.88, 157.12, 153.78, 134.79, 134.98, 127.04, 125.78, 121.54, 120.64, 116.40, 116.14, 112.52, 114.20, 108.80, 67.52, 16.50; FAB-MS: 345 (M + H). Anal. Calc. for $C_{17}H_{14}BrNO_2$: C, 59.30; H, 4.06; N, 4.06; found: C, 59.20; H, 3.84; N, 3.70.

*6.4. General procedure for the preparation of compounds **10a–d***

A mixture of substituted 4-bromomethylcarbostyril (**8a–d**) (4.0 mmol), 4′-fluoro-aniline (4.0 mmol) in super dry xylene (20 ml) was refluxed on oil bath for 10 h (135–148 °C). After the completion of the reaction, the separated solid was filtered, washed with excess of cold ethanol, dried and crystallized from suitable solvent.

*6.4.1. 4-[(4′-Fluoro) anilinomethyl]-1-aza coumarin (**10a**)*

Yield: 68%; colorless crystals (acetic acid); m.p. 308–310 °C; IR (KBr): 1659, 3363 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$, $\delta$ ppm): 4.08 (s, 1H, $CH_2NH$), 4.60 (s, 2H, C4−$CH_2$), 6.59 (s, 1H, C3−H), 6.90–7.50 (m, 8H, Ar-H), 8.70 (s, 1H, N*H*CO); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$ ppm): 160.43, 158.17, 156.70, 145.10, 136.22, 133.10, 132.60, 126.30, 123.66, 119.12, 116.71, 116.50, 114.30, 114.21, 109.88, 63.24; $^{19}$F NMR (470 MHz, $CDCl_3$, $\delta$ ppm): −122.90 (s, 1F, C4′−F); FAB-MS: *m/z* 269 (M + H). Anal. Calc. for $C_{16}H_{13}FN_2O$: C, 71.63; H, 4.88; N, 5.22; found: C, 71.20; H, 4.48; N, 5.02%.

*6.4.2. 6-Chloro-4-[(4′-fluoro) anilinomethyl]-1-aza coumarin (**10b**)*

Yield: 72%; pale green crystals (acetic acid); m.p. 270–272 °C; IR (KBr): 1662, 3370 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$, $\delta$ ppm): 4.10 (s, 1H, $CH_2NH$), 4.44 (s, 2H, C4−$CH_2$), 6.48 (s, 1H, C3−H), 6.88–7.48 (m, 7H, Ar-H), 8.78 (s, 1H, N*H*CO); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$ ppm): 160.94, 158.48, 155.74, 148.17, 136.20, 133.18, 132.57, 126.10, 123.28, 115.40, 116.17, 116.08, 114.40, 114.32, 108.38, 63.54; $^{19}$F NMR (470 MHz, $CDCl_3$, $\delta$ ppm): −122.20 (s, 1F, C4′−F); FAB-MS: *m/z* 303 (M + H). Anal. Calc. for $C_{16}H_{12}FClN_2O$: C, 63.48; H, 4.00; N, 9.25; found: C, 63.57; H, 3.97; N, 9.07%.

*6.4.3. 7-Chloro-4-[(4′-fluoro) anilinomethyl]-1-aza coumarin (**10c**)*

Yield: 70%; colorless crystals (acetic acid); m.p. 264–266 °C; IR (KBr): 1659, 3369 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$, $\delta$ ppm): 4.18 (s, 1H, $CH_2NH$), 5.08 (s, 2H, C4−$CH_2$), 6.38 (s, 1H, C3−H), 6.80–7.90 (m, 7H, Ar-H), 8.76 (s, 1H, N*H*CO); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$ ppm): 161.08, 158.70, 156.18, 146.24, 136.78, 133.30, 132.16, 126.10, 123.24, 118.92, 116.24, 116.16, 114.40, 114.20, 108.40, 63.28; $^{19}$F NMR (470 MHz, $CDCl_3$, $\delta$ ppm): −123.21 (s, 1F, C4′−F); FAB-MS: *m/z* 303 (M + H). Anal. Calc. for $C_{16}H_{12}FClN_2O$: C, 63.48; H, 4.00; N, 9.25; found: C, 63.50; H, 3.97; N, 9.10%.

*6.4.4. 8-Methyl-4-[(4′-fluoro) anilinomethyl]-1-aza coumarin (**10d**)*

Yield: 82%; pale green crystals (acetic acid); m.p. 230–232 °C; IR (KBr): 1657, 3357 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$, $\delta$ ppm): 2.48 (s, 3H, C8−$CH_3$), 3.72 (s, 1H, $CH_2NH$), 4.38 (s, 2H, C4−$CH_2$), 6.59 (s, 1H, C3−H), 6.88–7.46 (m, 7H, Ar-H), 8.76 (s, 1H, N*H*CO); $^{13}$C NMR (75 MHz, $CDCl_3$, $\delta$ ppm): 162.08, 158.80, 156.47, 144.38, 136.20, 133.14, 132.34, 126.17, 123.77, 118.78, 116.36, 116.28, 114.26, 114.18, 108.80, 63.44, 18.33; $^{19}$F NMR (470 MHz, $CDCl_3$, $\delta$ ppm): −123.89 (s, 1F, C4′−F); FAB-MS: *m/z* 283 (M + H). Anal. Calc. for $C_{17}H_{15}FN_2O$: C, 72.32; H, 5.36; N, 9.92; found: C, 71.94; H, 5.07; N, 9.70%.

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